INVESTIGATIONS INTO THE RELATIONSHIPS OF STRESS AND LEAF HEALTH OF THE GRAPEVINE (*VITIS VINIFERA* L.) ON GRAPE AND WINE QUALITIES

by

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Declaration of Originality

"This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright."

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Abstract

Trials were established to investigate links between late season leaf health and wine quality in Pinot Noir and Sauvignon Blanc, on a vineyard in Northern Tasmania.

One series of trials located areas within Pinot Noir and Sauvignon Blanc vineyards with regions of pre-existing shoot vigour variation. Ground-based measurements of vine canopy along with aerial imaging of near infrared and red light reflectance were used to characterise the canopies, and compare vigour assessment systems compared. Leaf health was assessed by chlorophyll concentration and late season retention. Yield and fruit chemistry attributes were assessed, and small-scale winemaking was used to investigate fermentation rate and wine quality.

Four nitrogen application trials were established: a nitrogen by irrigation trial (2005-06); a nitrogen rate (0, 20, 35 and 50 g N/vine in 2006-07 and 0, 20 and 50 g N/vine in 2007-08) by timing (pre-bloom, post bloom, pre-veraison and post veraison) trial; a nitrogen by exposure trial (nil or 100 g N/vine, shaded or exposed clusters) and a comparison of wine from must nitrogen increase by field fertiliser application or winery supplementation. Changes to shoot growth, leaf chlorophyll and retention, fruit yield and chemical attributes, and wine fermentation and composition were assessed.

Shoot vigour was correlated with leaf chlorophyll concentration and late season leaf retention in vigour trials, however leaf health measures could be influenced by nitrogen application timing independent of shoot vigour. Shoot growth responded to nitrogen availability prior to fruit set, while leaf chlorophyll was altered by nitrogen availability at any stage. Pre-bloom additions increase shoot growth in year 1 but in year 2 there was no impact from nitrogen timing. Leaf retention was improved by nitrogen applications after fruit set, but not before, and was independent of preceding season applications.

Pinot Noir fruit did not vary in total soluble solids (TSS) as a result of vine vigour. Sauvignon Blanc juice TSS from high vigour vines decreased, while titratable acidity increased in high vigour vines in all trials. Nitrogen application increased TSS in one season, while TA was higher after high rates of nitrogen, particularly when applied post bloom. Yeast assimilable nitrogen (YAN) influenced fermentation rates, and increased with vigour, and could also be increased by nitrogen addition. Wine tannins decreased with vigour and high rates of nitrogen, with changing sunlight exposure being the major influence. DAP additions produced wines that were distinct from field applied nitrogen, and when applied prior to inoculation led to an increase in wine colour density.

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1 Introduction

Grapevine canopy growth has a major impact on wine quality, and the most important component part of the canopy on wine quality is the leaves (Smart, 1985).

For the purpose of this thesis, vine leaf health is defined as "the ability of the leaf to carry out photosynthesis at a maximum rate with respect to leaf age and vine seasonal factors". The ability of a leaf to carry out photosynthesis will initially increase, until reaching a maximum level, after which it will invariably decline with age (Bertamini and Nedunchezhian, 2002, Petrie et al., 2000a, Poni et al., 1994), and in temperate climates undergo seasonal leaf senescence and dormancy (Wermelinger, 1991). Bertamini and Nedunchezhian (2002) found that leaves aged 40-50 days had a greater level of photosynthetic activity than both older leaves (90-100 days) and younger leaves (20-30 days). The maximal level of photosynthesis and the timing and rate of decrease will vary in response to a range of environmental influences including nutrient availability (Bertamini et al., 2002, Cook, 1966, Keller, 2004, Treeby, 2005, Ussahatanonta et al., 1996), water stress (Bertamini et al., 2006, Escalona et al., 1999, Escalona et al., 2002, Flexas et al., 2000, Kadam et al., 2006, Pellegrino et al., 2005, Silvestroni et al., 2005) and disease (Espinoza et al., 2007).

In an ideal canopy, most leaf expansion occurs in the early part of the growing season (Smart and Robinson, 1991), therefore late season leaf health is determined by the maximum level of photosynthesis attained by the leaf, and the rate of senescence progression. Senescence is a controlled process, associated with a number of specific genes (Hörtensteiner and Feller, 2002, Lim et al., 2007, Gan and Amasino, 1997). It has several important roles to play within plants, but one of the most important roles is to give plants the ability to recycle and redistribute nutrients (Thomas et al., 2003). It also allows plants to match their leaf area to the suitability of the environment for growth.

Senescence

Recycling of nutrients occurs on a seasonal basis, and senescence in grapevines involves the mobilisation of large amounts of nitrogen and other nutrients from shoots and leaves at the end of the growing season to be stored in permanent parts of the plant. It is then available for remobilising the following spring (Wermelinger, 1991, Wermelinger and Koblet, 1990). Within the annual cycle of growth and reuse of resources, senescence progression can also be triggered and accelerated by a number of environmental stresses. These include temperature extremes, water stress, ozone damage, pathogen damage or the impact of nutrients, and of these water stress and nitrogen deficiency are two of the most common promoters of senescence (Gan and Amasino, 1997).

Two different systems for describing the senescence process have been proposed, although neither is incompatible with the other. Noodén et al. (1997) describe three stages of senescence – initiation, degradation and then a final terminal stage of cell death. An alternate model is to break senescence down into "reversible" and "irreversible" stages (Thomas et al., 2003).

The initiation of senescence in a leaf is characterised by a transition from being a nutrient sink to being a nutrient source, and is accompanied by a decrease in photosynthetic activity (Yoshida, 2003). Senescence commences in the chloroplast, and the term gerontoplast has been coined to describe the aged, senescing chloroplast (Parthier, 1988, Hörtensteiner and Feller, 2002, Gan and Amasino, 1997). Chloroplast degradation is under nuclear control (Gan and Amasino 1997). Reversibility of this

process has been demonstrated in tobacco plants (Zavaleta-Mancera et al., 1999a, Zavaleta-Mancera et al., 1999b), prompted by removal of non-senescing plant parts combined with the application of cytokinin.

Nitrogen in particular will be remobilised during senescence (Masclaux et al., 2000). The chloroplast holds the majority of the protein in the leaf (Matile, 1992), with the major protein being ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), which has been nominated as the most abundant protein in the world (Ellis, 1979). Rubisco can contain up to 50% of the soluble protein in a leaf, and 20-30% of the total leaf nitrogen (Feller et al., 2008). Rubisco is relatively easily broken down (Mae et al., 1983), making it an easily remobilized nitrogen source in a leaf. As a result, while its primary function is the fixation of CO_2 in the Calvin cycle, Rubisco may also be utilised as a nitrogen store (Cheng and Fuchigama, 2000). It is suggested that it has the advantage over other nitrogen storage forms by also providing some, albeit small, increase in photosynthetic capacity (Stitt and Schulze, 1994). Its breakdown during senescence yields a range of amino acids that can then be transported to other organs in the plant, such as younger leaves, growing shoots and fruit, or stored in permanent parts of the plant (Feller et al., 2008).

The reduction in Rubisco leads to a reduced capacity within the leaf for photosynthesis. This can lead to a number of photoinhibitory responses, including the breakdown of the chlorophyll complex (Hörtensteiner, 2006).

Nitrogen uptake and assimilation

Nitrogen is particularly important in regulating senescence. More than 40% of vine leaf nitrogen is recycled (Wermelinger, 1991). As little as 1.25% of the nitrogen in a vine is lost when the leaves fall at the end of the season.

Vine nitrogen levels can frequently limit growth. By volume, nitrogen is the major plant nutrient taken up from the soil, and is second only to carbon in its abundance in plants. Nitrogen is available in soil in a range of forms. Nitrogen is primarily available in aerated soils in a nitrate form, although ammonium will also be present (Britto and Kronzucker, 2005, Keller et al., 1995, Miller et al., 2007b). The nitrogen in available forms may represent only 5% of the total soil nitrogen, with the remainder involved in soil microbe metabolism, and unavailable for uptake (Schulten and Schnitzer, 1998, Jackson et al., 2008). This large pool of nitrogen is dynamic, with mineralization of organic nitrogen forms leading to ammonia production increasing nitrogen availability for plants, and assimilation of ammonia and nitrate into organic forms reducing plant nitrogen availability. These are all microbially mediated processes. Further, bacteria are important in fixing atmospheric nitrogen to increase the total nitrogen content of the soil, or alternatively can drive denitrification processes under water logged conditions leading to loss of nitrogen back to the atmosphere (Schulten and Schnitzer, 1998).

Nitrogen uptake

As with many other plants, grapevines are able to utilize both nitrate and ammonium, as well as amino acids and urea, however nitrate is the main form taken up (Wermelinger, 1991, Roubelakis-Angelakis, 1991) and can be accumulated to high levels within vacuoles via a nitrate-H^+ exchanger (Lea and Azevado, 2006). Plants have evolved complex nitrogen uptake strategies, and are able to take nitrogen up from a large range of soil solute nitrogen concentrations, which can range from below 1 μM to over 1 M (various in Britto and Kronzucker (2005)). Plant roots have both high and low affinity nitrate transport systems (HATS and LATS respectively). The nitrate LATS are inducible

(iLATS), and there are both inducible and constitutive HATS (iHATS and cHATS respectively) (Miller et al., 2007b). HATS uptake is used for concentrations from 1 μM to 1 mM, while LATS are active above 0.5mM (Jackson et al., 2008). The iHATS uptake can react rapidly to increased nitrogen; uptake may increase within a few hours in some cases (Siddiqi *et al.* 1990 in (Britto and Kronzucker, 2005)), with the genes being expressed within minutes following exposure to nitrate (various in (Forde, 2002)). Local signalling pathways lead to increased growth of fine lateral roots and root hairs and reduce the growth of larger roots, and an increased ability to take up and assimilate nitrogen (Hare et al., 1997, Kakimoto, 2003, Miller et al., 2007b, Mok and Mok, 2001, Sakakibara, 2006, Stitt et al., 2002, Werner et al., 2001, Zhang and Forde, 2000, Forde, 2002, Forde and Lea, 2007, Takei et al., 2001, Takei et al., 2002). The intensity of this response is increased in plants with a low nitrogen status (Forde, 2002).

Nitrate and ammonium uptake will decrease with increasing plant nitrogen status (Keller et al., 1995). Glutamate, glutamine, arginine and nitrate itself have all been implicated as having the potential to down-regulate the uptake of nitrate (Britto and Kronzucker, 2005). Plants take up nitrogen at an increasing rate throughout the day, peaking towards the end of the day, with the greatest changes being on days with high sunlight (Glass, 2003). It is probable that this is related more to tissue sugar levels, and a greater ability to drive nitrogen assimilation, than it is to do with transpiration (Hunter and Ruffner, 1997). Nitrate uptake can continue through the night, when it may fall by only 30% of the daytime rate (Stitt et al., 2002). When external nitrate and ammonium concentration is high (>1mM), LATS are used for uptake. Nitrate LATS, similarly to the iHATS uptake used at low concentration, is downregulated by exposure to nitrate (Siddiqi *et al.* in (Britto and Kronzucker, 2005)). Ammonium LATS are not down-regulated by internal nitrogen levels and to avoid toxicity, plants will start a corresponding ammonium efflux (Britto and Kronzucker, 2005).

Mycorrhizal fungi may also assist plants take up nitrogen, along with many other nutrients (Jackson et al., 2008, Schreiner, 2004, Oaks et al., 1991). They are able to access pores that are too small for the plant roots, and are more plastic in their morphology particularly for immobile nutrients. Therefore they may have a role to play in the more immobile ammonium uptake and also possibly in the uptake of nitrogen from organic nitrogen sources (Jackson et al., 2008).

Nitrogen assimilation

Following uptake, nitrogen reductase is used to assimilate nitrogen into amino acids. Keller (2004) states that when carbohydrate supply is high and soil nitrate supply is low, nitrate assimilation takes place largely in the roots, and is then transported via the xylem, primarily as glutamine. Zerihun and Treeby (2002) found that as nitrogen uptake increased, the majority of the NR activity occurred in the leaves, where rates are over double that of the roots. This is supported by the work of Hunter and Ruffner (Hunter and Ruffner, 1997), who also found that NR activity changed with leaf height and age, and by Keller et al. (1995). Root NR activity also varies seasonally, being most active during periods of root growth (Hunter and Ruffner, 1997, Jackson et al., 2008). Using nitrate to total nitrogen levels of xylem sap, Peuke (2000) conclude that between 40% and 75% of nitrate reduction occurred in the shoots. Nitrate reduced in upper plant parts may be transported to the roots in the phloem and then returned in the xylem (Roubelakis-Angelakis, 1991). Glutamine can be carried back to the roots via the phloem, where the adjusted glutamate:glutamine ratio will have a feedback effect by limiting nitrate uptake and NR activity (Campbell, 1999).

Nitrate reductase activity in grapevines has been shown to peak around mid morning to midday, decreasing again to a low level in the late afternoon. NR activity in leaves is stimulated by red light (Hunter and Ruffner, 1997), and this combined with an increased activity in warmer temperatures (Smart, 1991) leads to less activity in shaded leaves. NR is present in leaves in the early morning, and is stimulated by the early morning light (Stitt et al., 2002), As noted above, nitrate uptake is lower in the morning than in the afternoon, and at this time nitrate assimilation can be occurring at twice the rate of uptake (Stitt et al., 2002). The NR activity also exceeds the nitrogen flux through the GOGAT pathway, leading to an accumulation of ammonium and glutamine (Stitt et al., 2002).

Nitrate assimilation is a multistep process. The nitrate taken up from the soil must be reduced to ammonium (Roubelakis-Angelakis, 1991), the first step being the reduction of nitrate to nitrite by nitrate reductase (NR):

$$NO_3^- + 2H^+ \rightarrow NO_2^- + H_2O$$

This process uses NADH/NADPH as an electron donor (Campbell, 1999), and occurs in the cytosol (Oaks et al., 1991).

Nitrite is toxic, so this step rapidly moves on to the next phase, where the nitrite gets further reduced to ammonium by nitrite reductase (NiR):

$$NO_2^- + 8H^+ \rightarrow NH_4^+ + 2H_2O$$

Ammonium is also toxic to plants, and most is rapidly incorporated into amino acids for further metabolism or transport (Keller, 2004). The major pathway for this involves glutamine synthetase (GS) and glutamine synthase (also known as glutamine-2-oxoglutarate aminotransferase – GOGAT). Firstly, glutamate reacts with the ammonium to form glutamine, catalyzed by GS:

$$NH_4^+ + \text{Glutamate} \rightarrow \text{Glutamine}$$

Ammonium for this reaction may come from ammonium taken up by roots, the reduction of nitrate or secondary metabolism, such as photorespiration (Forde and Lea, 2007).

The second section of the process takes 2-oxoglutarate, supplied by photosynthetic metabolism, and glutamine, and converts that into two units of glutamate.

$$\text{Glutamine} + \text{2-oxoglutarate} \rightarrow 2\ \text{Glutamate}$$

One unit of glutamate is then returned to the GS catalyzed reaction, while the other can be used to build further amino acids (Keller, 2004, Oaks et al., 1991, Bernard and Habash, 2009).

An alternative pathway used in the assimilation of ammonium exists, where the enzyme glutamate dehydrogenase (GDH) catalyzes the following reaction:

$$\text{2-oxoglutarate} + NH_4^+ \rightarrow \text{Glutamate} + H_2O$$

GDH is stress related, and can assist by assimilating ammonium in tissues where levels are excessive. Conversely, because it is reversible it can be used to oxidize glutamate to provide carbon skeletons for continued ATP regeneration, and remobilize proteins, when photosynthesis is limiting (Pageau et al., 2006, Miflin and Habash, 2002). GDH is particularly active in mitochondria (Miflin and Habash, 2002).

Nitrogen transport

Nitrogen can be transported around a vine in a number of forms, including nitrate in the xylem, ammonium and amino acids in both xylem and phloem. The major constituent in the xylem is the free amino acids (Roubelakis-Angelakis, 1991).

Early in the season, the major xylem-transported amino acid is glutamine (Roubelakis-Angelakis, 1991, Sponholz, 1991, Stines et al., 2000). Glutamine appears to be the major nitrogen transport form in the phloem. In one study, heading in to flowering and fruitset, the glutamine levels decreased and aspartic acid levels rose. In later berry development, glutamine and aspartic acid levels maintained an equilibrium (Sponholz, 1991). Alanine and proline are also be involved in transport. Another study found that glutamine levels made up 85% of the xylem nitrogen in the sap of Müller Thurgau vines (Keller et al., 2001b), a result repeated by (Peuke, 2000) in Riesling. There are differences between species of *Vitis* regarding the forms of amino acid most commonly found in the sap, as well as compared to the hybrids. Rootstock will also influence vine amino acid composition (Sponholz, 1991, Holzapfel et al., 2001). Arginine has also been found to be a major transport form of nitrogen besides inorganic forms, accompanied by glutamine and glutamic acid, as well as aspartic acid (Wermelinger, 1991).

Storage

Nitrogen may be stored in a number of forms, including nitrate and as amino acids, particularly arginine (Roubelakis-Angelakis, 1991, Sponholz, 1991, Stines et al., 2000). Arginine has a high nitrogen to carbon ratio (4:6) and is an important storage form of nitrogen within many higher plants (Forde and Lea, 2007). Arginine is used to store nitrogen in the berry, and is often the major amino acid in grape berries (Bell and Henschke, 2005). Arginine is also a precursor for polyamines (Alcázar et al., 2006, Forde and Lea, 2007), which can have an important role in plant stress response. Nitrogen stored as Rubisco has been noted by other researchers (Cheng and Fuchigama, 2000).

Aminotransferase reactions can be used to produce a number of other amino acids from the α-amino group on the glutamate molecule. Glutamate itself may also be converted into γ-aminobutyric acid (GABA), which can accumulate following exposure to a number of stresses on a plant. Shelp *et al.* 2006 (Forde and Lea, 2007) have also found evidence of GABA being a signal compound. Another important glutamate conversion is to arginine and proline.

Nitrogen effects on photosynthesis

The primary role of a leaf is the harvesting of light energy via photosynthesis, and approximately 60-80% of leaf nitrogen can be invested in the photosynthetic apparatus (Kumar et al., 2002). It is therefore not surprising that nitrogen nutrition can have a major impact on the rate of photosynthesis. Nitrogen content is strongly tied to photosynthesis and to senescence as a contributor to Rubisco as well as other proteins, and low nitrogen availability can limit Rubisco levels (Mae et al., 1983, Warren and Adams, 2004). Nitrogen is also linked to photosynthetic activity through a requirement for carbon and for energy for its assimilation. Therefore, a nitrogen deficit can lead to reduced photosynthesis; conversely, a reduction in photosynthesis can lead to reduced levels of nitrogen assimilation.

Nitrogen and Cytokinins

Nitrogen levels will alter plant cytokinin concentration. Cytokinins are plant hormones, consisting of a group of compounds which play a number of important roles to in

plants (Sakakibara, 2006). Cytokinins have traditionally been considered as root hormones, however there is evidence that they are produced in other tissues also (Sakakibara, 2006, Lombard et al., 2006). They are produced very rapidly following changes in nitrogen availability (Takei et al., 2001), with one cytokinin production pathway specifically triggered by nitrate, and another responding to both nitrate and ammonium (Sakakibara, 2006). Cytokinins, nitrate and glutamate have the ability to act as long-range signalling compounds to signal nitrogen availability throughout the plant (Forde, 2002, Forde and Lea, 2007, Takei et al., 2001, Takei et al., 2002, Sakakibara, 2006).

Stress-associated hormones such as ethylene, abscisic acid (ABA) and salicylic acid have been observed to rise in association with senescence, although their roles are not yet clear (Lim et al., 2007). ABA is important as a signal mechanism for low water status (Dry and Loveys, 1998, Hartung et al., 2005, Jiang and Hartung, 2007, Wasilewska et al., 2008), which has a known impact on promoting senescence (it is not proven yet that abscisic acid is a cause or a symptom of senescence (Lim et al., 2007)). Water stress is also known to cause reduced nitrogen uptake, which will also promote senescence (Keller, 2004, Fanizza et al., 1997).

Nitrogen deficiencies can stimulate senescence processes (Crafts-Brandner et al., 1998, Diaz et al., 2006, Hörtensteiner and Feller, 2002, Keller et al., 1998). As noted above, senescence will mobilise nitrogen to supply organs that require it. Conversely, high levels of nitrogen availability can delay senescence, particularly as a result of increased cytokinin production (van Doorn, 2008, Lim et al., 2007, Sakakibara, 2006, Gan and Amasino, 1995), and lead to increased growth of shoots and leaves (Sakakibara, 2006, Hare et al., 1997).

The impact of different levels of nitrogen availability on vegetative growth is summarised in Figure 1-1.

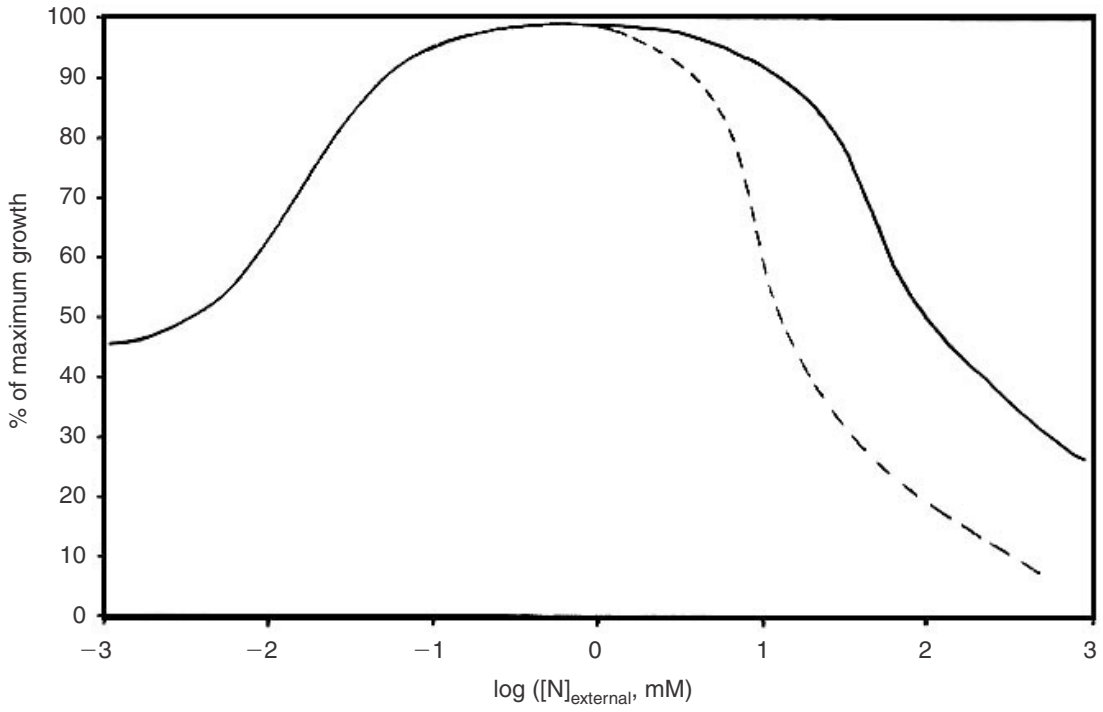

Figure 1-1 Generalised growth curve in response to nitrogen addition. The dotted line representes ammonium and the solid line nitrate. (Ammonium toxicity occurs at a lower level than nitrate toxicity). Adapted from Clement et al., 1978 by Britto and Kronzucker (2005).

Grapevine annual nitrogen cycle

Bud burst – remobilisation of stored nitrogen

Nitrogen is stored in the trunk, shoots and roots of a vine over winter. There is a very intense period of remobilisation of these nitrogen reserves in the period from sap bleed up until bloom (Wermelinger, 1991). One study determined that until there were around 5-6 leaves, all the nitrogen used in growth was from remobilised reserve stores (Wermelinger, 1991). One reason for this is that in some environments, soil temperatures can lag behind air temperature increases, so growth of roots is delayed also. The total N mass in the woody structures can decrease by up to 76% The younger wood is the main source of this nitrogen, with shoots using nitrogen first from canes, then trunks, and finally roots (Wermelinger, 1991). There is evidence that remobilised root stored nitrogen is primarily utilised in the roots, while shoot growth utilises nitrogen stored in above ground parts of the vine (Araujo and Williams, 1988).

Flowering and fruitset

Nitrogen content in the young inflorescences starts at the same level as that of vegetative tissue and slowly decreases as the season progresses, while partitioning to growing grape berries begins after bloom (Wermelinger, 1991).

Nitrogen demand of growing shoots

Shoot nitrogen content decreases steadily after budbreak, with the rate of decrease falling as veraison is approached. Following veraison, the shoot nitrogen increases again. Nitrogen peaks at the end of the vegetative growth phase, due to the large amounts of remobilised nitrogen in the phloem.

Nitrogen demand of ripening fruit

Fruit growth on grapevines involves three distinct phases. Phase I follows fertilisation of the ovule, and involves an increase in berry size by cell division. Phase II is known as the lag phase, when berry growth is minimal. This is followed by phase III, the start of which coincides with veraison. Berry expansion resumes, and berries become a major sink for carbohydrates (Petrie et al., 2000b).

There are two phases of intense nitrogen incorporation, matching the maximum growth rate stages of berry development. The first is in the couple of weeks preceding the "pea-size" stage, and the second starts at the beginning of maturation and lasts for around two weeks. Towards the end of fruit ripening, large amounts of free amino acids (primarily arginine) are transported from the roots to the berries, with a further increase in total and soluble nitrogen (Wermelinger, 1991).

A number of grape varieties will also import a large amount of proline into the fruit (Schaller, 2005). Proline levels in berries measured throughout the growing season in Riesling increased along with sugar levels. It was thought that this was in response to potassium accumulation, with proline acting as a compatible solute, and protecting proteins and membranes against osmotic damage. In this situation, it was postulated that the proline reflected the total influences of stressors on the plant. The level of accumulation of proline is varietally distinctive, and Pinot Noir is not a large accumulator of nitrogen (Schaller, 2005).

Nitrogen re-absorption for storage ready for following season budburst

Nitrogen store replenishment begins as early as fruit maturation, and reserves continue to increase after harvest until the end of leaf fall (Wermelinger, 1991). Nitrogen destined for storage is sourced both from soil uptake, and from the degradation of nitrogenous compounds during senescence.

The period between harvest and leaf fall is particularly important in replenishment of stored vine nitrogen (Conradie, 2004). Higher levels of cropping can decrease the stored nutrient levels in the wood (Balasubrahmanyam, 1978)

Nitrogen deficiencies are a common cause of stress in vineyards (Keller, 2004). Nitrogen will be naturally limiting in soils with low organic matter content (Löhnertz, 1991, Conradie and Saayman, 1989), when the total amount of soil nitrogen cycling through the microbial mineralization – immobilization pathway is decreased. Nitrogen is lost from the vine as a result of pruning and fruit harvest (Conradie, 1991), and may also be lost from the vine root zone soil through leaching (Schaller, 1991). Uptake from the soil may be reduced by competition from other plants, such as inter-row grasses (Morlat and Jacquet, 2003, Chantelot et al., 2002). Reduced soil moisture, which limits root growth and the movement of solutes through the soil, can also influence uptake, and will reduce leaf nitrate reductase activity (Keller, 2004). Increasing nitrogen availability to vines with a nitrogen deficit will lead to higher bud burst (Kliewer et al., 1991), greater shoot growth with more leaves (Bell and Robson, 1999, Candolfi-Vasconcelos et al., 1997, Grechi et al., 2007, Shawky et al., 2004), larger leaves (Shawky et al., 2004) and increased leaf chlorophyll (Candolfi-Vasconcelos et al., 1997, Shawky et al., 2004). Nitrogen added in excess has also been demonstrated to reduce growth (Bell and Robson, 1999).

Nitrogen management in the vineyard

Determining optimal vine nitrogen management has long been a challenge to viticulture focussed on wine grape production. Early studies of nitrogen impact on vines were largely focussed on impact on yield (Kliewer and Cook, 1974, Cook, 1966), however nitrogen levels will impact more than just yield, and may alter fruit attributes that impact wine quality. Leaf photosynthesis can be increased by nitrogen addition (Syvertsen, 1987, Vasconcelos et al., 2005), and nitrogen addition can increase grape sugars as a result (Treeby et al., 2000). Conversely, actively growing shoots and leaves may also represent an alternate sink to the fruit for resources. This can occur as a result of excessive nitrogen application (Conradie, 2001). The number of leaves present, and their distribution, will determine what proportion of light reaching the vine will enter the fruit zone, which can have a major impact on grape quality (Smart, 1985).

Nitrogen management is further complicated by the difficulty in assessing what is available to the vine. Soil nitrogen tests generally reflect either the total nitrogen (of which most immobile) or a snapshot of nitrate nitrogen, which will be in constant flux. Mineralization is greatly affected by environmental conditions, and is maximal between 25°C and 35°C, with soil moisture at field capacity (Knoepp and Swank, 2002). Changes in these factors will reduce how much nitrogen is made available to plants by the soil bacteria. It is unsurprising that measurements of soil nitrogen in vineyards have not found good correlations with vine nitrogen levels (Löhnertz, 1991).

Determining the level of adequacy of vine nitrogen concentration is the first stage in that process, and can be done using several methods. There are a range of different standards, timings and plant organs that are recommended for sampling (Kliewer, 1991, Robinson, 1992). Diagnosis should also incorporate an assessment of the vine canopy

and vigour level (Robinson, 1992). Leaf chlorophyll may have potential use as an indicator of vine nitrogen (Spring and Jelmini, 2002).One method used to determine nitrogen requirements is to calculate the loss of nitrogen through removal and calculate a replacement requirement. Conradie (1991) found that for every ton of grapes removed from a vineyard, an average of 1.6 kg of nitrogen is also removed, with a range of results between 1.4 kg/ton and 1.9 kg/ton. Therefore, a crop yielding 8 ton/ha would require a replacement of between 11 and 15 kg N/ha. A unit of nitrogen added to the soil does not necessarily equate to that much nitrogen being made available to the plant however, due to the assimilation by soil microbes, and loss to other plants.

Nitrogen applied at different growth stages can induce different responses in the vine (Bettiga and West, 1991, Conradie, 1991, Conradie, 2001, Holzapfel and Treeby, 2007, Peacock et al., 1991). Nitrogen uptake is increased in the period of time between the end bloom to veraison, and again from harvest until leaf fall (Conradie, 1991, Peacock et al., 1989). In many regions nitrogen applications post harvest are recommended, to coincide with the late-season uptake period and to increase nitrogen storage for the following season (Peacock et al., 1989, Bettiga and West, 1991, Conradie, 2004, Conradie and Saayman, 1989). This requires that an active canopy to be present at the time of application to allow nitrogen uptake to occur, which may be an issue in cool climate regions where there can often be poor leaf health at harvest (Bettiga and West, 1991, Peacock et al., 1991).

Late season leaf health links to grape and wine attributes

Leaf health can be linked to grape quality in a number of ways. Photosynthesis in the leaves supplies the sugars required for ripening of the fruit, and for vine growth (Maroco et al., 2002, Petrie et al., 2000a, Keller, 2010). If the leaf area is not able to produce sufficient sugars to meet the demands of the fruit, ripening can be delayed. In cool climate viticulture this may lead to an inability to ripen fruit to a suitable level, therefore poor leaf health may have a negative effect on wine quality. A leaf area to fruit ratio that is too low can lead to a reduction in the sugar accumulation in fruit (Petrie et al., 2000b), although once a suitable leaf area is reached further leaf area increases do not provide additional increases in fruit sugar levels (Jackson, 1986).

As noted above, the regulation of senescence involves a number of signals to the vine, including the levels of nitrogen compounds and hormones such as cytokinin and abscisic acid (ABA). ABA rises with stress and with senescence (Dry and Loveys, 1998, Hartung et al., 2005, Jiang and Hartung, 2007, Wasilewska et al., 2008), leading to decreased shoot tip activity and activating senescence processes. Cytokinin hormones not only delay senescence, they also promote shoot tip growth (Gan and Amasino, 1997). Cytokinin production increases when there is a high nutrient availability and reduced when the vine faces nutrient deficiency or water stress (Sakakibara, 2006). Therefore, conditions that reduce leaf senescence and raise leaf chlorophyll will also encourage vigorous shoot growth and increased leaf numbers, while conditions that may accelerate the onset and progression of senescence may also reduce shoot growth. The exception to this is senescence that is a result of leaf shading (Gan and Amasino, 1997). In the case of vineyards, this generally occurs when shoot growth is excessive (Smart, 1985), so senescence in this situation indicates too much growth, not too little.

Increasing vigour leads to longer shoots with more laterals and larger leaves (Smart, 1985), which can alter the vine canopy microclimate, by progressively increasing shading and reducing air movement within the canopy. Dense canopies can impact product quality by leading to higher disease levels (English et al., 1989), lower fruit set (Dry, 2000) and reduced phenolic concentration (Cortell and Kennedy, 2006, Cortell et al., 2008).

Conversely, canopies with insufficient vigour may have excessive fruit exposure, which can cause damage from increased berry temperature and radiation, as well as reduced yields (Dry, 2000). Low vigour vines have a reduced leaf area compared to vines with a higher vigour (Kliewer and Dokoozlian, 2005, Smart, 1985, Winkler, 1970).

The factors that impact on leaf health can similarly impact overall vine growth. When water and nutrients are abundant, vine leaf health may be at a maximum, and canopy growth can also be high (Keller, 2004), leading to dense canopies with limited light penetration (Smart, 1985).

Grapes react to sunlight by producing a protective layer of ultra-violet light absorbing phenolic compounds, including compounds such as tannins that are also of major importance to winemaking. Tannins provide protection from oxidation (Ginjom et al., 2010), bind anthocyanins to stabilise colour (Fulcrand et al., 2006, Cheynier et al., 2006), and contribute largely to the mouth feel of wine (Gawel, 1998).

Fruit set and yields

Decreases in light penetration as a result of increased vigour will alter the microclimate in the fruit zone, (Smart, 1985) and air movement (Thomas et al., 1988). Dry (2000) reviewed the influence of canopy growth on yield and fruitfulness, and conclude the canopy impact on light penetration will impact fruit set, since increases in both light exposure and temperature of the buds were positively correlated to fruitfulness. Other researchers have also noted that both low light and reduced airflow may lead to reduced fruit set (Dry and Loveys, 1998, Smart and Robinson, 1991). Conversely, nutritional deficiencies and vine stress, which will reduce vine vegetative growth, may also lead to poor fruit set (Ussahatanonta et al., 1996) , and under optimal weather conditions nutrient stress may be the primary determining factor if fruit set (Ewart and Kliewer, 1977).. Therefore, it may be concluded that the highest yields are attained by canopies that are neither too vigorous or too stressed.

This has been observed in several studies that looked at yield and vigour relationships. In some cases yield increased with vigour, (Proffitt et al., 2006, Clingeleffer and Sommer, 1995), while in other circumstances, yield decreased or remained static (Cortell et al., 2007b).

Major components of yield include shoots per vine, bunches per shoot, and fruit set, and all may be affected by nitrogen availability. The response to nitrogen will vary according to the original nitrogen status of the vine. Under conditions of deficiency, an addition of nitrogen can increase yields (Kliewer et al., 1991, Kliewer and Cook, 1974, Bell and Robson, 1999), through increased bud burst, fruitfulness and fruit set (Keller et al., 1998, Kliewer et al., 1991, Keller et al., 2001a, Spayd et al., 1993, Ewart and Kliewer, 1977). Under some situations, berry size may also be affected (Holzapfel and Treeby, 2007, Spayd et al., 1993).

Sugars, pH and Titratable Acidity

Additional nitrogen supplied to vines with low nitrogen availability can lead to increases in leaf health or leaf area (Keller et al., 2001b, Vasconcelos et al., 2005). This can lead to increased sugar accumulation, and increases in grape soluble solids after nitrogen fertilisation have been observed by some authors (Bell and Henschke, 2005, Vasconcelos et al., 2005). However, the increase in shoot growth that can be stimulated by nitrogen application can lead to the growing shoots acting as a competitive sink for vine carbohydrates (Smart, 1991), and this can lead to a delay in sugar accumulation in the fruit. Excess availability of nitrogen that leads to delays in maturation has been observed

in a range of trials (Delgado et al., 2004) .

Berry exposure can increase total soluble solids (TSS), although if excessive it may delay sugar accumulation (Bergqvist et al., 2001). This is not a very dependable response however, and Cortell et al. (2008) report that increasing vigour did not consistently alter TSS levels. More consistently observed is an increase in titratable acids with higher vigour (Cortell et al., 2008). Malic acid degradation will be increased by greater fruit temperature (Keller, 2010), which will lower titratable acidity measurements. Potassium movement into the fruit can also increase in higher vigour vines as a result of increased shading (Smart, 1985, Keller, 2010), which will raise pH at a given titratable acidity (Jackson, 1994, Keller, 2010). Reduced temperature can decrease the degradation of malic acid in the fruit, leading to increased titratable acidity in shaded fruit (Keller, 2010, Cortell et al., 2008, Bergqvist et al., 2001). Even though acid concentration is increased, pH may not drop, and can even increase due to the malic acid, and also as a result of increased influx of potassium cations. Wine pH can influence both the mouthfeel of a wine (Keller, 2010), and also the wine colour (Somers and Evans, 1977).

Yeast Assimilable Nitrogen

Yeast assimilable nitrogen (YAN) consists of ammonium and free amino acids, and these will rise with increasing nitrogen availability. YAN levels are considered deficient when below 140 mg/L (Butzke, 1998, Bell and Henschke, 2005), and deficient YAN in musts and juice can lead to stuck or sluggish fermentations (Monteiro and Bisson, 1991, Henschke and Jiranek, 1993, Alexandre and Charpentier, 1998, Julien et al., 2000, Cramer et al., 2002) and the production of hydrogen sulphide (Henschke and Jiranek, 1993, Jiranek et al., 1995b, Hallinan et al., 1999, Gardner et al., 2002). This deficient level will be modified by yeast strain (Jiranek et al., 1995b, Gardner et al., 2002, Bell and Henschke, 2005). YAN levels up to 500 mg/L have been shown to continue to increase fermentation rates. YAN deficiency prior to inoculation will decrease yeast cell biomass, which can occur both through increases in the metabolism by each yeast cell (Beltran et al., 2005, Monteiro and Bisson, 1992), and also by increasing biomass when nitrogen is made available (Beltran et al., 2005, Bisson, 1991, Gardner et al., 2002, Monteiro and Bisson, 1992, Varela et al., 2004).

Hydrogen sulphide can be generated when the pathway to produce the amino acids cysteine and methionine is interrupted due to a lack of *O*-acetyl homoserine and *O*-acetyle serine, and their production will decrease under low nitrogen conditions (Gardner et al., 2002, Hallinan et al., 1999, Jiranek et al., 1995b, Stratford and Rose, 1985). In that scenario, hydrogen sulphide will accumulate in the cell before diffusing in to the wine (Bell and Henschke, 2005). Hydrogen sulphide production generally decreases when nitrogen is added to the must (Bell and Henschke, 2005, Jiranek et al., 1995b), although this will not always related to the H2S in the wine, and may not be a consistent trend (Ugliano et al., 2009).

Nitrogen availability may drive vine growth, and higher vigour vines will often have a higher tissue nitrogen concentration (Hall et al., 2002). The link is so strong that one proposed measure of vine nitrogen status is must arginine levels (Bath et al., 1991, Kliewer and Cook, 1974). Arginine is also one of the major amino acids contributing to yeast assimilable nitrogen (Bell and Henschke, 2005, Henschke and Jiranek, 1993). Therefore, increasing yeast assimilable nitrogen with increasing vigour is expected, and has been observed (Cortell et al., 2008).

Disease risk

Dense canopies can also lead to increased incidence of disease (Mundy and Beresford, 2007, English et al., 1989, Thomas et al., 1988), and also to reduced yields due to shading in the fruiting zone (Dry, 2000).

Phenolic compounds have important roles to play as sun protection compounds and also in combating disease. Therefore, reductions in phenolics due to high nitrogen levels can leave fruit more vulnerable to sun damage and infections such as botrytis (*Botrytis cinerea*) and powdery mildew (*Uncinula necator*) (Keller et al., 2003). However, even more crucial may be the changes in microclimate within the canopy (Mundy and Beresford, 2007). Increasing canopy density can also increase disease risk further, due to the increase in humidity, as well as difficulties in reaching those areas when applying antifungal agrochemicals.

Increased vigour can increase the occurrence of infection by fungal pathogens such as botrytis, due to the higher leaf area in the fruit zone decreasing air movement through the fruit zone, which increases drying time of dew or precipitation, and decreases the coverage of preventative sprays (Thomas et al., 1988, English et al., 1989, Smart, 1991). Further increasing this susceptibility is reductions in stilbene concentration, a group of phenolic compounds which have antifungal properties (Adrian et al., 2000, Keller, 2010), and are also produced in response to UV light.

Phenolics and Tannins

A number of grape phenolic compounds are altered by vine vigour, particularly in response to light levels. Light, in particular in the ultra violet range, will stimulate the production of flavan-3-ols (Cortell and Kennedy, 2006, Keller et al., 2003, Spayd et al., 2002) and other phenolic compounds (Adrian et al., 2000, Bergqvist et al., 2001). Tannins are formed when flavan-3-ol compounds condense, and their levels are similarly influenced by exposure to light (Ristic et al., 2007). As a result, increasing vine vigour will reduce fruit exposure and so result in a decrease in these compounds (Cortell et al., 2005). Flavan-3-ols and tannins are very important to red wine quality, being a major component determining wine astringency (Gawel, 1998) and binding to anthocyanins to stabilise colour in red wines (Fulcrand et al., 2006, Somers and Evans, 1977).

Studies examining the impact of vine canopy growth on phenolics have found that increasing vigour produces grapes with lower tannin concentration (Cortell et al., 2005, Cortell et al., 2008, Smart, 1985, Lamb et al., 2004).

Anthocyanins

Anthocyanin concentration is less affected by shading than phenolics (Cortell and Kennedy, 2006, Ristic et al., 2007), however the composition of the constituent anthocyanins is likely to change (Cortell and Kennedy, 2006, Keller and Hradzina, 1998). Anthocyanins, which form the basis of the red colour in red wine, are sensitive to changes in grape temperatures (Spayd et al., 2002, Bergqvist et al., 2001, Deis et al., 2009, Ristic et al., 2007). Increasing temperatures from 15°C to 25°C (Deis et al., 2009) can lead to an increase in anthocyanins, with a maximum production around 30°C (Spayd et al., 2002). Berry temperatures above 35° will decrease anthocyanin production, which may occur on sun-exposed fruit in warm climates (Bergqvist et al., 2001, Spayd et al., 2002).

A number of studies have identified that the anthocyanin composition is particularly affected by vine vigour (Cortell et al., 2007a, Cortell et al., 2007b), although anthocyanin concentration is not linearly related to vigour (Guidoni et al., 2008, Cortell et al., 2007a,

Cortell et al., 2007b, Rustioni et al., 2009). Anthocyanins form the basis of colour in red wines, as free anthocyanin in young wines, and bound either by polymerisation to compounds such as tannins, or by copigmentation in older wines (Cheynier et al., 2006, Gao et al., 1997, Romero-Cascales et al., 2005). Anthocyanin concentration and colour density has been positively correlated to wine quality (Jackson et al., 1978).

Analysis of grape tissue in culture (Pirie and Mullins, 1976), and of field trials on vines (Keller and Hradzina, 1998) indicate that there is also a direct decrease in the production of both anthocyanins and phenolics from increased nitrogen availability.

Nitrogen levels available for yeast metabolism are also of importance, and deficiencies have been linked to wine fermentation problems and reduced wine quality (Bell et al., 1979, Henschke and Jiranek, 1993, Jiranek et al., 1995a).

Wine Aroma

Grapes contain many compounds that will contribute to the aroma of wine. A number of these have been shown to be influenced by vine nutrition and shading (Joscelyne et al., 2007, Choné et al., 2006, Peyrot des Gachons et al., 2005, Ristic et al., 2007). Fermentation of grape must will also contribute to the odour of wine, forming the fermentation bouquet.

Wine can contain a number of esters, several of which are derived from the metabolism of amino acids. Esters contribute a range of aromas to the wine, and together can contribute a general fruitiness that positively influences wine quality (Bell and Henschke, 2005, Guitart et al., 1999). Several esters are produced by the breakdown of amino acids (Garde-Cerdán and Ancín-Azpilicueta, 2007) and also through synthesis using other nitrogen sources (Miller et al., 2007a). As a result, increases in vineyard nitrogen will increase the esters in wine (Ough and Lee, 1981, Webster et al., 1993, Giorgessi et al., 2001, Beltran et al., 2005, Linsenmeier et al., 2005, Miller et al., 2007a, Bell and Henschke, 2005). Nitrogen availability has also been demonstrated to impact the levels of higher alcohols in wine (Beltran et al., 2005, Gallander et al., 1990, Giorgessi et al., 2001, Webster et al., 1993). Higher alcohols at low rates can have a positive impact on wine quality, but a negative impact as levels increase. Higher alcohol production will initially be decreased as YAN is increased in a deficient ferment medium, but will increase again as YAN levels increase above moderate levels (Bell and Henschke, 2005). The complexity of higher alcohol production has led to a range of different results from nitrogen application either to the must (Beltran et al., 2005, Guitart et al., 1999, Hernández-Orte et al., 2002, Ugliano et al., 2008) or in the vineyard (Gallander et al., 1990, Giorgessi et al., 2001, Webster et al., 1993).

An improved understanding of the links between leaf health and its links to positive and/or negative attributes of grapes and wine quality may allow improved decision-making in vineyards. Leaves have one attribute that makes them excellent as an indicator of vine status, in that they are visually very obvious. This can allow visual assessments to be made rapidly, however assessments are only useful if the ramifications of leaf health are understood.

The trials outlined in this thesis aim to improve the understanding of how leaf health can be linked to wine quality. The first section of the thesis details a series of trials established on sites where leaf health varied naturally because of changing vine vigour, in Pinot Noir and Sauvignon Blanc. By observing vines with such a pre-existing difference in leaf health and characterising the canopy attributes, yield and fruit and wine characteristics, the trials aimed to demonstrate leaf health links to all stages of the winegrape-growing process. Leaf health could also be linked to other aspects of canopy

growth, to determine what correlations there are between the different canopy measures.

The second section of the thesis describes a series of trials where leaf health was manipulated by addition of nitrogen fertilisers to vines on a block with relatively uniform vine vigour. The first stage of this trial was designed to confirm that nitrogen application was more important than soil moisture, since both may have a major impact on leaf health. Subsequent trials applied different rates of nitrogen at different application dates over multiple seasons to vines with poor late season leaf health and assessed the impact on canopy growth, yield, fruit and wine characteristics. The project aimed to further develop an understanding of leaf health links to fruit and wine. Improved information on nitrogen fertiliser addition in cool climate Pinot Noir viticulture was also sought from this trial, along with studies to investigate differences between vineyard nitrogen applications and nitrogen addition to fermenting musts on wine quality.

2 General Materials and Methods

2.1 Site Summary

All trials were established on the Tamar Ridge Estate's Kayena vineyard in northern Tasmania, a cool climate viticulture area. The vineyard sits at a latitude of 41°11'S and a longitude of 146°53'E.

The nearest Bureau of Meteorology weather station (Low Head; station number 091293) records a mean annual temperature of 16.1°C, and the nearest rain gauge (Beaconsfield; station number 091001) records an annual average rainfall of 943.6 mm (Bureau of Meteorology (www.bom.gov.au/climate/data/)).

2.2 Season summaries

Data was collected across four seasons – 2005-06, 2006-07, 2007-08 and 2008-09. The monthly average rainfall and monthly average temperatures are provided below (Figure 2.2-1, Figure 2.2-2, Figure 2.2-3, Figure 2.2-4), and annual averages are given in Table 2.2-1. Rainfall for the 2005-06 season was above average, with a large amount of winter rain. 2006-07 was below average, particularly throughout the growing season. Rainfall was also below average in 2007-08 and 2008-09. Temperatures in all seasons were above average.

Table 2.2-1 Annual average temperatures and average rainfall, and long-term averages. Rainfall average from Beaconsfield (Bureau of Meteorology station 091001) from 1901-2011. Temperature average - Low Head (Bureau of Meteorology station 091293) from 1997-2010.

Season	*Rainfall (mm)*	*Temperature (°C)*
2005-06	1041	16.4
2006-07	500	16.8
2007-08	787	16.8
2008-09	752	16.2
Long term average	944	16.1

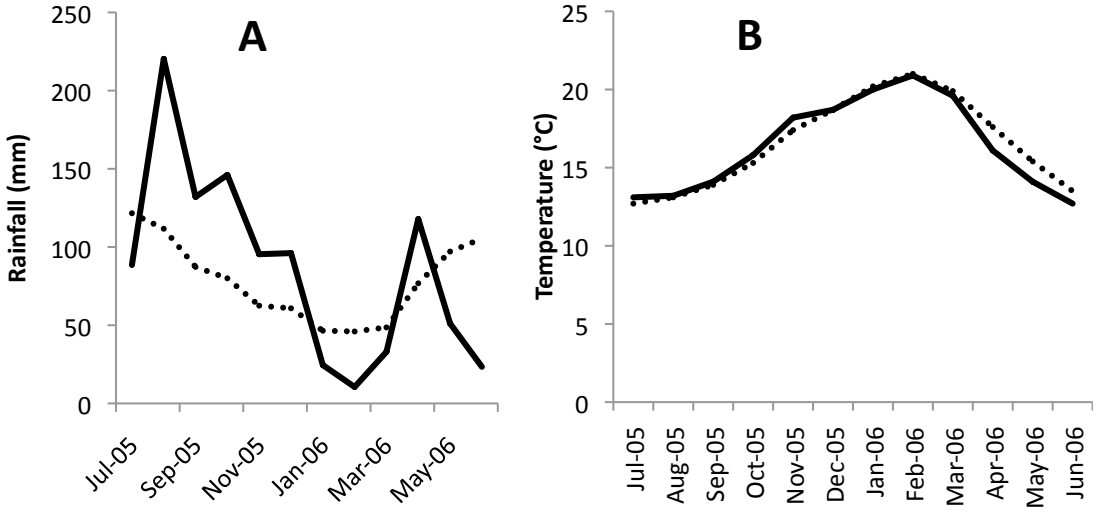

Figure 2.2-1 Monthly rainfall (A) and temperature (B) averages, 2005-06. Rainfall data – Beaconsfield (station number 091001); Temperature data - Low Head (station number 091293). All data from Bureau of Meteorology

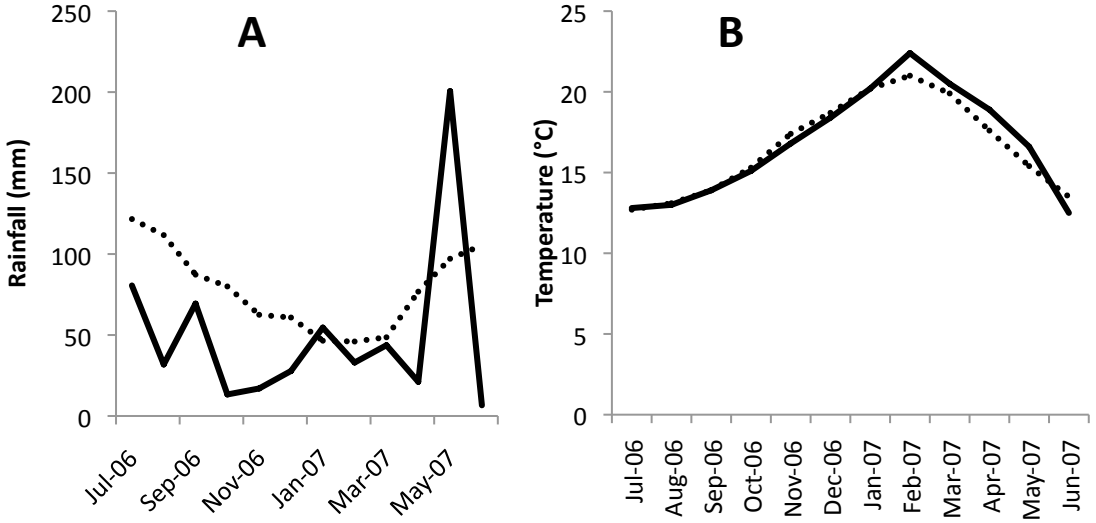

Figure 2.2-2 Monthly rainfall (A) and temperature (B) averages, 2006-07. Rainfall data – Beaconsfield (station number 091001); Temperature data - Low Head (station number 091293). All data from Bureau of Meteorology

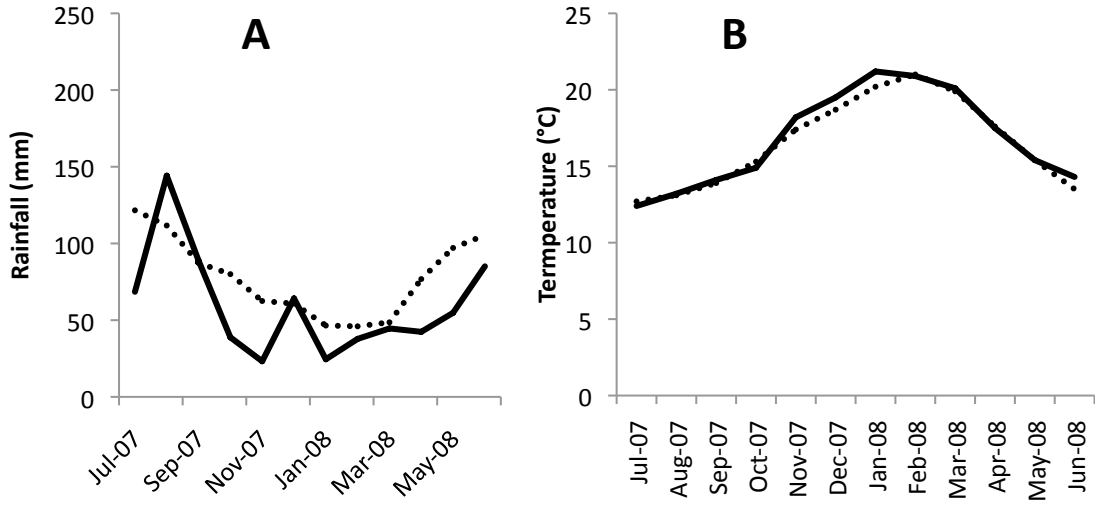

Figure 2.2-3 Monthly rainfall (A) and temperature (B) averages, 2007-08. Rainfall data – Beaconsfield (station number 091001); Temperature data - Low Head (station number 091293). All data from Bureau of Meteorology

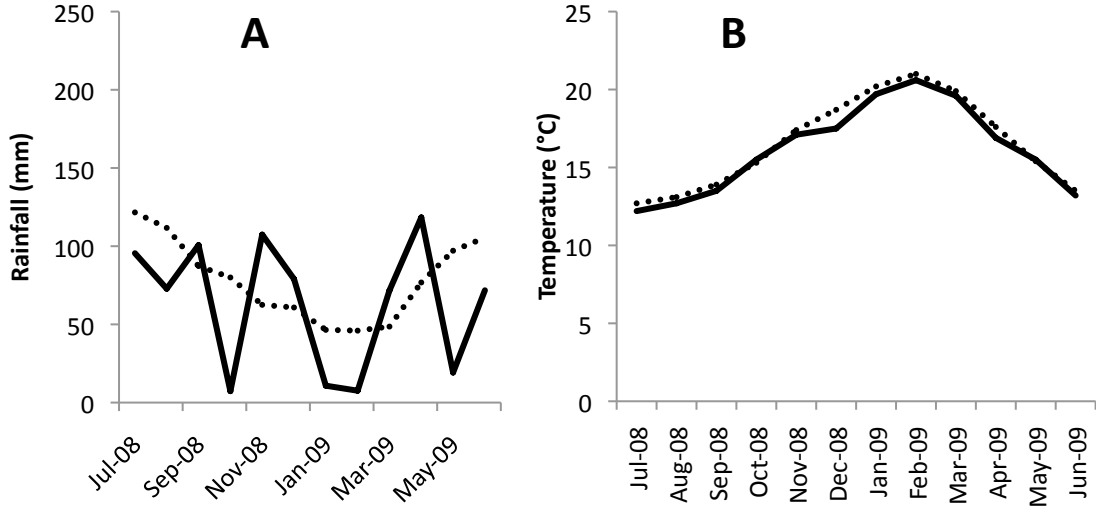

Figure 2.2-4 Monthly rainfall (A) and temperature (B) averages, 2008-09 Rainfall data – Beaconsfield (station number 091001); Temperature data - Low Head (station number 091293). All data from Bureau of Meteorology

2.3 Trial layouts

Vigour gradient trials

Three blocks were selected that had a wide range of vigour levels across a small area, identified using aerial infrared imaging (detailed below). One block was Sauvignon Blanc (clone unknown), and two were Pinot Noir clone G5V15. The trial was conducted over three years, commencing in the 2005-06 season. All trials were drip irrigated and all vines were own-rooted.

One trial was located in each of the Pinot Noir blocks (block A and block B). The Pinot Noir blocks were both cane pruned on a Scott Henry trellis. One of these sites, block A, was investigated in 2005-06, 06-07 and 07-08, while the other, block B, was only used in 2005-06.

All blocks had vines spaced 1.5 m apart and rows 2.25m apart. The trellising system used a post every four vines and the four vines between the posts made a single panel, which formed the basic measurement unit for all trials.

In block A Pinot Noir, four zones were identified, straddling four rows. Aerial vigour maps were used to identify the location of each panel (Figure 2.3-1). Each row was treated as a replicate for statistical analysis. The vigour zones were numbered from 1 (low vigour) to 4 (high vigour). The same vines were used in all three years of the trial.

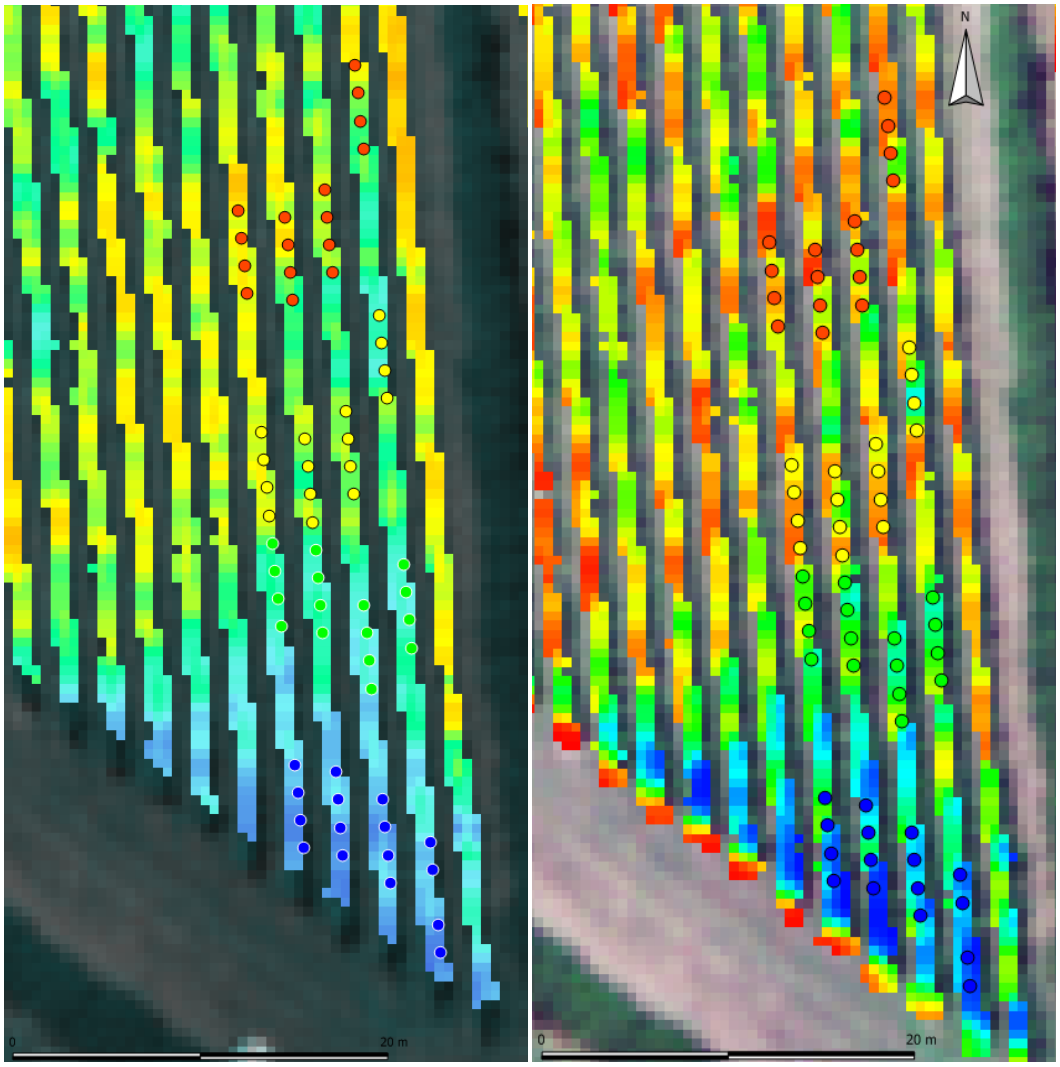

Figure 2.3-1 Vine-only PCD map of block A in 2006 (left) and 2007 (right), where red indicates low vigour and blue indicates high vigour. Measurement vines are marked (Red = vigour class 1 (low vigour), yellow = vigour class 2, green = vigour class 3 and blue = vigour class 4 (high vigour)).

Block B Pinot Noir was divided into five vigour zones, numbered from 1 (low vigour) to 5 (high vigour) (Figure 2.3-2). Four replicates were selected for each vigour zone.

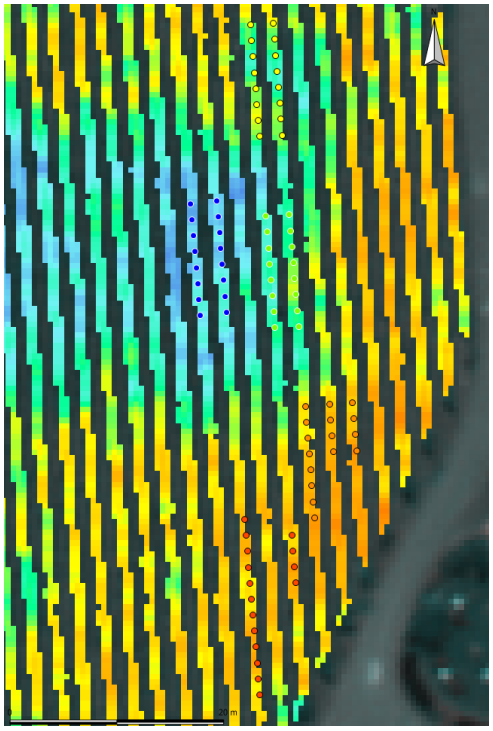

Figure 2.3-2 Block B vine-only PCD-generated vigour map from 2006, where red indicates low vigour and blue indicates high vigour. Measurement vines are marked (Red = vigour class 1 (low vigour), orange = vigour class 2, yellow = vigour class 3, green = vigour class 4 and blue = vigour class 5 (high vigour)).

Two Sauvignon Blanc trial sites were identified in 2005-06 to compare the impact of changing vine vigour on this variety (Figure 2.3-3). One trial used vines trained to a Scott Henry trellis (block C-SH) and the second trial used vines that were cane pruned on a vertical shoot positioned (VSP) trellis (block C-VSP). All Sauvignon Blanc trial sites each contained two levels of vigour. These were classed as "low" and "high". Each vigour zone level had six replicates.

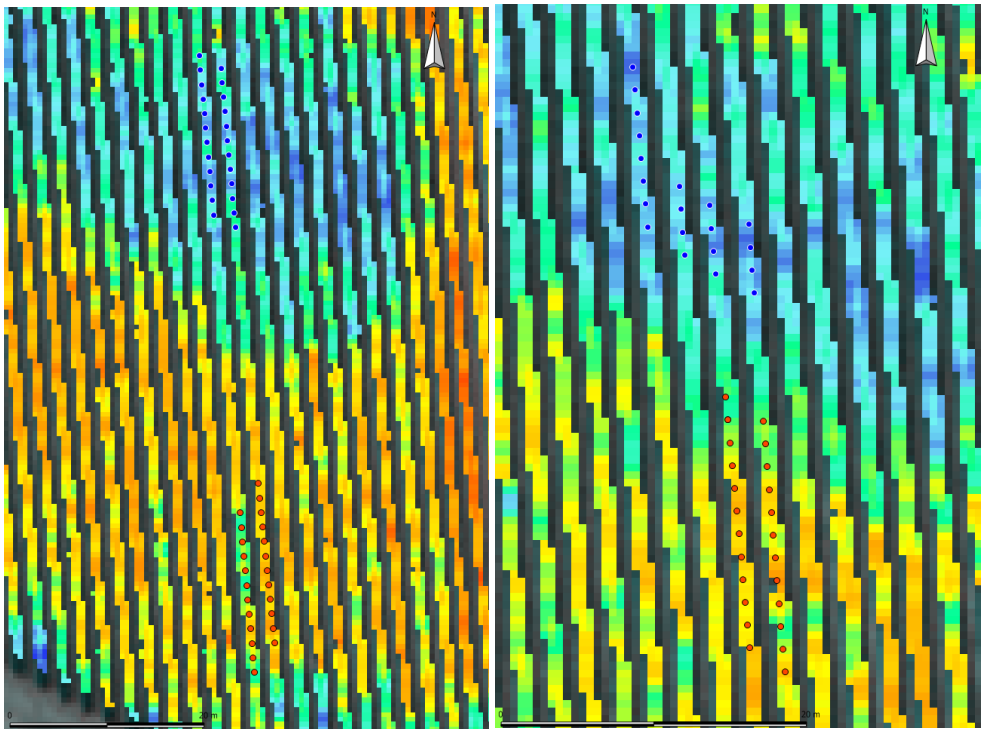

Figure 2.3-3 Sauvignon Blanc vine-only PCD-generated vigour map from 2006, where red indicates low vigour and blue indicates high vigour. Vines on left are trained to a VSP trellis, and on the right to a Scott Henry trellis (block C-SH). Measurement vines are marked (Blue = high vigour, red = low vigour)

A new section of the same Sauvignon blanc vineyard block was selected to conduct the vigour trial in 2006-07 (block C-07), again with two vigour zones, "low" and "high" (Figure 2.3-4). All vines were trained to a Scott Henry trellis. The new area was selected because it demonstrated a greater vigour range than the sites chosen in the previous season.

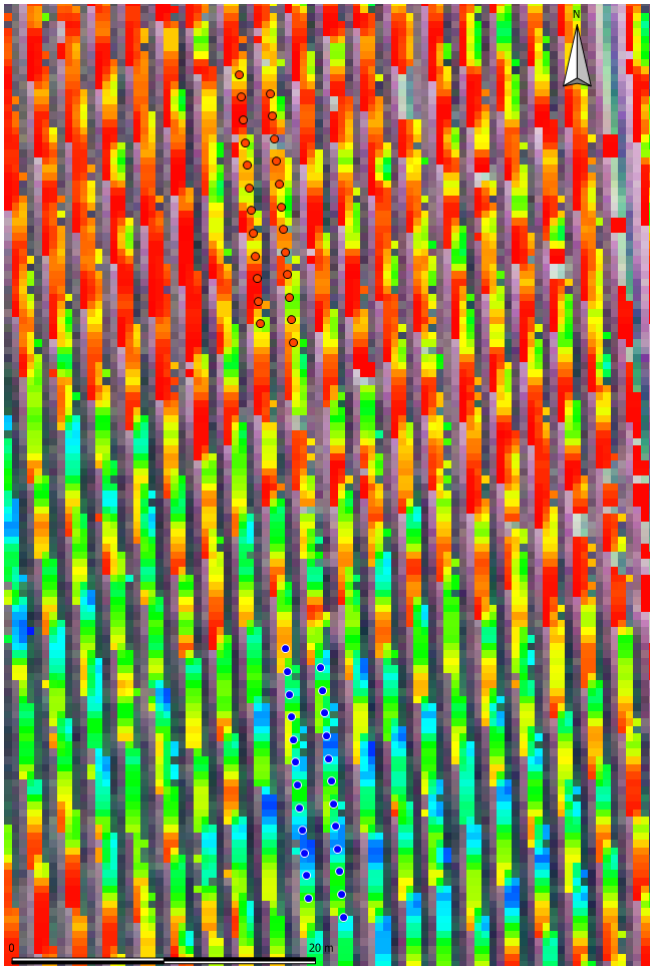

Figure 2.3-4 Sauvignon Blanc vine-only PCD-generated vigour map from 2007, where red indicates low vigour and blue indicates high vigour. Vines are trained to a Scott Henry trellis. Measurement vines are marked (Blue = high vigour, red = low vigour)

A schedule of analyses, along with results, for the vigour trials are outlined in section 3 (commencing p. 39).

Selection of vigour zones

Aerial vigour maps were obtained from Specterra Services (see p. 25). Plant Cell Density (PCD) values were assigned colours using Specterra's proprietary colour scheme, to allow clear identification of different vigour levels. Colours are generated by the PCD values.

These maps (Figure 2.3-1, Figure 2.3-2, Figure 2.3-3, Figure 2.3-4) were then used as the base for selecting areas with different vigour levels.

Nitrogen application trials

All nitrogen application trials were within the one vineyard block of *Vitis vinifera* cv. Pinot Noir, clone 114, on Tamar Ridge Estates' Kayena vineyards in northern Tasmania. The vines were own-rooted, cane pruned on a Scott Henry trellis and drip irrigated.

Irrigation management was controlled by the vineyard managers.

The trellis construction had a post every four vines. These four vines formed an individual panel, and each treatment described below was applied to all vines within one panel. The central two vines were then used as monitor vines for all canopy measurements.

Nitrogen application rate by irrigation trial

A pilot study was established in 2005-06 to assess the relative importance of nitrogen and water in contributing to the early senescence. It was established in mid February 2006 during veraison, and all vines received uniform management up until that point. The trial was established as a randomised complete block design with four replicates.

Nitrogen was added at three different rates: 0 g N/vine ("Control"); 17 g N/vine (50 kg N/ha) and 51 g N/vine (150 kg N/ha). Irrigation was applied at three different levels: nil; standard vineyard practice; and triple standard volume. These were applied as a factorial trial, for a total of nine treatment combinations per replicate. All other vineyard operations were applied across all vines in the trial.

Nitrogen was applied as urea. It was pre-weighed and then broadcast on the ground by hand, with most falling in the herbicide sprayed strip beneath the vines. Urea, as used in 2005-06, requires hydrolysis and then conversion to nitrates to make it available, a process that will be initiated rapidly when soil moisture is present (Carter and Vlek, 1983). The urea was broadcast prior to rain, allowing hydrolysis and conversion to nitrate to take place.

Standard irrigation in this block was delivered using drip-line with integrated 1.6 L/hr pressure compensated emitters, spaced 750 mm apart. Increased irrigation rates were achieved by adding two extra pressure compensated 2 L/hr emitters in the dripper line to each pre-existing emitter. Blocking off all integral dripper emitters for the dripper line in the treatment panel, using standard electrical style tape, created the nil irrigation treatments.

Berry sampling was carried out prior to harvest to monitor maturity. All treatments were harvested on the same date, when berry samples indicated that the juice density was over 23° Brix. Local winemakers indicated this was a target grape Brix level for commercial table wine production.

In the following season, vines were tracked to see what impact the previous season's nitrogen had on subsequent vine behaviour. Three monitor shoots were selected per monitor vine, one from the distal end of the cane, one from the middle of the cane and one close to the trunk. All monitor shoots were on the upper cordon arms, trained upwards. The monitor shoots were marked and used for shoot length and EL-stage assessments, leaf counts, node counts and leaf chlorophyll estimation.

Nitrogen timing by rate trial

As a result of the response to nitrogen in the pilot study, an expanded nitrogen trial was established and operated for two seasons, 2006-07 and 2007-08. Four different nitrogen timings were used: pre-bloom, post bloom, pre-veraison and post veraison. In 2006-07, nitrogen application rates of either 20, 35 or 50g N/vine (equivalent to 59, 104 and 148 kg N/ha respectively each date) were applied at each timing. In 2007-08, applications were repeated at all four dates for the 20 g N/vine and 50 g N/vine treatments. In 2006-07 five plots were left unfertilised as a control, and in 2007-08 the number of control panels was expanded to 13 to increase the statistical strength. The layout for the trial was

a completely randomised design, with each treatment combination being replicated five times.

Nitrogen was applied as Easy N®, a commercial liquid fertiliser produced by Incitec Pivot (Southbank, VIC). EasyN® is a blend of urea and ammonium nitrate dissolved in water, and delivering 42.5% N (w/v), comprising of 50% urea, 25% ammonium and 25% nitrate. Applications were made to the area under the vineyard's drip irrigation system's wetted area, to ground that was lightly disturbed to ensure it remained where it was added until the next irrigation.

Three monitor shoots were selected per monitor vine, one each from the distal section of the cane, one from the middle of the cane and one close to the trunk. All monitor shoots were on the upper cordon arms, trained upwards. These were marked and revisited for a number of the regular monitoring procedures carried out through the season (detailed below).

Nitrogen addition by bunch exposure

Established in the 2006-07 season, this trial utilised 5 panels of vines treated with a high rate of nitrogen (100 g n/vine) pre-bloom (30th November 2006), and 5 panels of vines with no nitrogen addition. This rate was selected to assess the impact nitrogen at levels significantly in excess of most vineyard application rates. The layout was a completely randomised design, and nitrogen application was in the form of EasyN®.

Bunches were graded based on their position in the canopy and whether they were exposed to sunlight ("external") or shaded by leaves ("internal"). The internal and external bunches were then harvested separately.

Field-applied nitrogen compared to winery-applied nitrogen

This trial was established in the 2007-08 season. Sixteen panels were selected randomly within the vineyard block. From these 16 panels, five were randomly selected to receive nitrogen fertilisation, and the remainder were unfertilised. The number of treated and untreated panels was designed to produce enough fruit for a minimum of five ferments from fertilised vines and a minimum of 12 ferments from unfertilised vines.

Nitrogen was applied to field nitrogen treatments as a split application of 20 g N/vine post bloom and a further 20 g N/vine pre-veraison, for a total of 40 g N/vine. These dates were used because studies in 2006-07 had indicated there would be an increase in YAN (Figure 4.3-6) with minimal change in vine growth (Table 4.2-23). The rate was selected to target a YAN level of at least 250 mg N/L. Selecting a target must YAN is not simple, since different yeast strains can have different nitrogen requirements (Gardner et al., 2002, Jiranek et al., 1995a), and there will be a pool of assimilable nitrogen in grape solids that will not be measured by juice YAN analysis (Bell and Henschke, 2005, Stines et al., 2000). Nonetheless, fermentation studies indicate that a ferment medium YAN in the range of 250 - 350 mg N/L is sufficient to allow ferments to complete fully while leaving little residual nitrogen (Henschke and Jiranek, 1993). Therefore, a juice YAN of 250 mg N/L was considered to be an appropriate target for this trial. In the nitrogen timing by rate trials in 2006-07, vines that received nitrogen applications at a rate of 20 g N/vine post-bloom had a must YAN of 233 mg N/L, 75 mg N/l more than the control vines (Figure 4.3-6). The same rate of nitrogen added pre-veraison increased the must YAN to 178 mg N/L, which was 20 mg N/L more than the control. It is recognised that nitrogen uptake will be influenced by soil nitrogen status (Keller, 2004), therefore post-bloom fertilisation may lead to altered uptake of fertiliser applied pre-veraison. Nonetheless, based on these results, it was decided that split

applications of 20 g N/vine at each date for a total of 40 g N/vine was likely to produce a YAN close to the target of 250 mg N/L.

Nitrogen was applied using Easy N® (see above for Easy N® product details – p. 23). Additions were made to soil that had been crumbled to a shallow depth to ensure none ran off the mound, and in a position to ensure irrigation from the vineyard's drip irrigation system would soak the region.

2.4 Canopy and Leaf Health Measurements

Vigour scorecard

A scorecard was constructed for this study to allow rapid objective assessments of the vine canopy. The vigour scorecard used attributes from the wine quality scorecard proposed by Smart and Robinson (1991), modified to score the canopy based on canopy density and leaf health, not suitability for making wine. The vigour scorecard is summarised in Table 2.4-1.

Table 2.4-1 Vine vigour scorecard used to assess post-veraison leaf health in Pinot Noir in 2005-06

Attribute	*Score*
Canopy gaps	
About 50% or more	1
About 40%	2
About 30%	3
About 20%	4
About 10% or less	5
Leaf Size	
Very small	1
Slightly small	2
Average	3
Slightly large	4
Very large	5
Leaf colour	
Unhealthy, marked necrosis or chlorosis	1
Mild nutrient deficiency symptoms	2
Yellowish green, healthy	3
Green, healthy, slightly dull and pale	4
Dark green, shiny, healthy	5
Canopy density (Mean leaf layer number)	
About 1 or less	1
About 1.5	2
About 2	3
More than 2	4
Fruit exposure	
About 60% or more exposed	1
About 50% exposed	2
About 40% exposed	3
About 30% exposed	4
About 20% exposed	5
Average leaf loss	
7-8 basal nodes bare	1
5-6 basal nodes bare	2
3-4 basal nodes bare	3
1-2 basal nodes bare	4
No leaves missing	5
Total Score	Sum

Remote sensing

SpecTerra Services Pty Ltd (Leederville, Western Australia) supplied digital multispectral images (DMSI), using plant cell density (PCD) as the vegetation index. PCD is a ratio of reflected near infrared radiation to reflected red radiation:

PCD = NIR/R (Dobrowski et al., 2002)

The near infrared waveband centred on 780 nm, and red light reflectance around 675

nm. Data in each waveband was collected as 14-bit digital information. The PCD was then calculated, using the formula (NIR/R)*512. The factor 512 was used as a multiplier to facilitate further processing in a 16-bit unsigned integer format. This PCD value was then converted to an 8-bit scale using a normalized quantization scaling algorithm. Over the ranges of PCD values present, this scaling is essentially linear. PCD data analysed in these trials was the 8-bit data, with a range fom 0 to 255.

SpecTerra's post processing involves the use of a proprietary algorithm designed to remove pixels that do not contain information from the vine row itself, eliminating interference from reflectance of the interrow surfaces.

Images were supplied as both coloured and grey-scale geotiff files. The coloured files were used to quickly discriminate areas of high and low vigour, while the grey-scale images were used to obtain the PCD values of measurement panels. Distance along the row to the measurement panels was used to select the pixels over the measurement panels, and PCI Geomatica's Freeview program (PCI Geomatics, Toronto - http://www.pcigeomatics.com/) was used to find the PCD value of the relevant pixels.

Leaf chlorophyll concentration

Chlorophyll levels were estimated using a CCM200 chlorophyll meter (Opti-Sciences, Hudson, NH, USA). The CCM200 meter measures transmission of light through a leaf at two wavelengths, 660nm and 940nm. 660nm is strongly absorbed by chlorophyll, while 940nm, which is not absorbed by chlorophyll, is used as a reference wavelength, to adjust for differences in leaf structure (Richardson *et al* 2002). The CCM200 does not provide an absolute measure of chlorophyll, instead calculating a proprietary Chlorophyll Content Index (CCI). A number of authors have found that the results from the CCM200 correlate well with chlorophyll concentration measurements (Li et al., 2007, Richardson et al., 2002, Rodrigo et al., 2007).

Each trial used different protocols for selection of leaves, and these are outlined in the relevant chapters.

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were made using an OS-30p (Opti-Sciences, Hudson, NH, USA) chlorophyll fluorescence meter. The OS-30p was set to work in Screening F_v/F_m mode. Leaves were dark adapted for 20 minutes using the supplied clips, and then the F_o, F_m and F_v/F_m levels were recorded. Each trial used different protocols for selection of leaves, and these are outlined in the relevant chapters.

Point Quadrat

A welding rod with a sharpened tip was used for point quadrat measurements. A one metre rule was suspended by wires from the foliage wires, and insertions were made every 100mm. This was repeated for three lengths of the rule, except where vine arms were missing and resulting gaps would have skewed data. Measurements were made in the fruiting zone - however, due to the necessarily linear nature of the rule, and the not-so-linear height of the fruiting zone, this was only an approximate region.

A portable voice recorder was carried, and for each insertion contact between the tip of the rod and vine parts was recorded. Categories that were recorded were leaf; yellow leaf (for a leaf with no visible chlorophyll); cluster; and gap, where no contacts were made. Contact with canes was ignored.

The analyses outlined in Smart and Robinson (1991) were used to provide average leaf

layer number, percent gaps, percent interior leaves and percent interior clusters. The calculations of Meyers and vanden Heuval (2008) were used to calculate occlusion layer, cluster exposure layer and leaf exposure layer. Yellow leaf counts were analysed separately.

Leaf light transmission

Sunlight transmitted through leaves was assessed using a LiCor LI250 light meter, recording μmol of photosynthetically active radiation. Leaves were selected that had a range of leaf chlorophyll concentrations. Leaf chlorophyll concentration was estimated as CCI units using a CCM 200 chlorophyll meter (Opti-Sciences, Hudson, NH, USA), in a marked spot on the leaf. This same area was then laid over the LiCor sensor, ensuring light was not able to pass between the leaf and the sensor. The sensor was aligned to be perpendicular to the sun, and light passing through the leaf was recorded. Unimpeded sunlight energy was also recorded each time a new leaf was sampled, and transmission was expressed as a percentage of this value.

This trial used leaves from the nitrogen timing by rate trial in 2007-08.

Leaf anthocyanin

Leaf anthocyanin was assessed by visual estimation. In 2006-07, the number of leaves were counted on each monitor shoot with more than 50% of the leaf veins displaying anthocyanin colouration.

In 2007-08 a scorecard was developed, where 1 = no red visible; 2 = some veins red; 3 = all veins red; 4 = All veins and some lamina red; and 5 = all lamina red. Vines were scored.

Leaf area measurements

Leaf area was assessed in the nitrogen timing by rate trial in 2006-07, using a model developed based on leaf vein lengths.

A number of leaves of a range of sizes were collected to determine the relationship between leaf vein length and leaf area. Grapevines have 5 major veins (Galet, 2000) and the lengths of all these were measured. The first major lateral veins either side of the main central vein are considered one set of veins, and the second pair of lateral veins considered as a second set.

The area of each leaf was determined using a flat bed scanner, with a card of known area included in the scan. The image was then exported as a jpeg file. Images were imported into ImageJ image analysis software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2009) and contrast increased to make the leaf blade area all black. This allowed for easy selection of the leaf area using ImageJ's magic wand selection tool. By firstly calibrating the image using the scanned card of known dimensions, we were able to determine leaf area.

This leaf area was then put into a spreadsheet alongside the vein lengths. Regression analysis was performed looking at leaf area against main vein length; main vein length + average of each set of lateral vein lengths; the squared value of the sum of all vein lengths; the main vein multiplied by the average of the first set of lateral veins and then by the average of the second set of lateral veins; and the main vein multiplied by the average of all the lateral veins.

Regression analysis indicated that the index that correlated best to actual leaf area calculations came from multiplying the main vein length with the average of the first set of laterals and then with the average of the second set of laterals. This was termed the "leaf area calculation index":

Leaf Area Calculation Index = A x $((B_1 + B_2)/2)$ x $((C_1 + C_2)/2)$

Where:

A = Mid vein length;

B = Vein length of secondary lateral veins

C = Vein length of remaining major vein branch

Field measurements involved measuring the length of all five major veins, and calculating the corresponding index.

Early season shoot growth

The modified E-L system proposed by Coombe (1995) was used to assess early season shoot growth stage. E-L measurements were made on either all shoots on a cane, or monitor shoots, as detailed in each chapter materials and methods sections.

Shoot tip growth

Shoot tip growth was measured using a scorecard system developed by Dr Richard Smart (pers. comm.). Tips are graded on a scale of 5, with the following values:

Table 2.4-2 Shoot tip growth scorecard

Score	***Description***
1	Shoot tip extends well beyond separated leaves below
2	Shoot tip close to separated leaf tips
3	Expanded leaf tips move past shoot tip
4	Multiple expanded leaves move past shoot tip
5	Shoot tip dehisced

The assessment involves wrapping a hand around a shoot and moving it towards the tip. This will make the leaves lie parallel to the shoot, with the leaf tips towards the shoot apex. As the hand moves towards the shoot tip, it becomes clear whether the leaves or tip extend further, and the score can be judged.

Shoot diameter

Shoot diameter measurements were made using a set of digital calipers. The measurement was made at the second clear internode. In cases where the cane was substantially elliptical in cross section, measurements were made at the widest and the narrowest points, and an average of the two values was recorded.

Pruning weights

Pruning weights were collected per vine, with the number of vines assessed varying between treatments (outlined in each trial's materials and methods section). Canes were counted before pruning, as were the number of arms. Vines were pruned to the standard vineyard practice of cane pruning on a four-arm Scott Henry trellis, with an average of around 10 nodes per arm. All pruned material of less than two years of age was then collected and weighed on electronic scales with a resolution of 10 g. Wood older than one season was not included.

Mean cane weight was calculated by dividing the total pruning weight by the cane weight count.

Canopy light penetration

Light entering the canopy of vines in the nitrogen timing by rate trial in 2007-08 was measured using a LiCor LI250 light meter, recording μmol of photosynthetically active radiation. Measurements were taken with a uniform layer of cloud reducing the intensity of direct sunlight. The sensor was placed inside the canopy, with its base on the cordon cane, ensuring that the operator was not in the sensor field of view. Measurements were taken at intervals of 30cm along the length of the cane cordons in the two measurement vines, starting from about 20cm from the tip of the cane. This gave 7-8 measurements per panel.

Canopy light penetration was assessed in 2007-08 in the nitrogen timing by rate trial, during March.

Results were assessed as averages of the raw data, and ANOVA's calculated as averages of the natural logs of the original data.

Periderm formation

The degree of periderm browning, or shoot maturation, was assessed visually as a change in colour of the shoot surface from green to brown. This was quantified by counting the number of internodes on each shoot assessed that were over 50% lignified.

Periderm browning was analysed in 2005-06 late in the season in the irrigation by nitrogen rate trial, and in 2006-07 in block A Pinot Noir in the vigour trial and the nitrogen timing by rate trial.

Leaf nutrient analysis

Full leaf tissue testing was conducted by a commercial laboratory (CSBP, Bibra Lakes, WA) on dried tissue samples collected in the 2006-07. Analysis included percentages of total nitrogen, phosphorus, potassium, sulphur, sodium, calcium, magnesium and chloride. In addition, copper, zinc, manganese, iron, nitrate and boron were assessed as mg/kg. Samples were sent from both lamina and petioles sampled mid January from control vines and vines receiving 50 g N/vine pre-bloom. Samples were taken at veraison from control vines and vines receiving 50 g N/vine pre-bloom, post bloom and pre-veraison.

Lamina samples were taken for veraison sampling, as opposed to petiole samples, to allow comparison with published standards (Robinson, 1992). Regression analysis indicated that total nitrogen concentration of lamina and petioles was closely correlated (Figure 2.4-1).

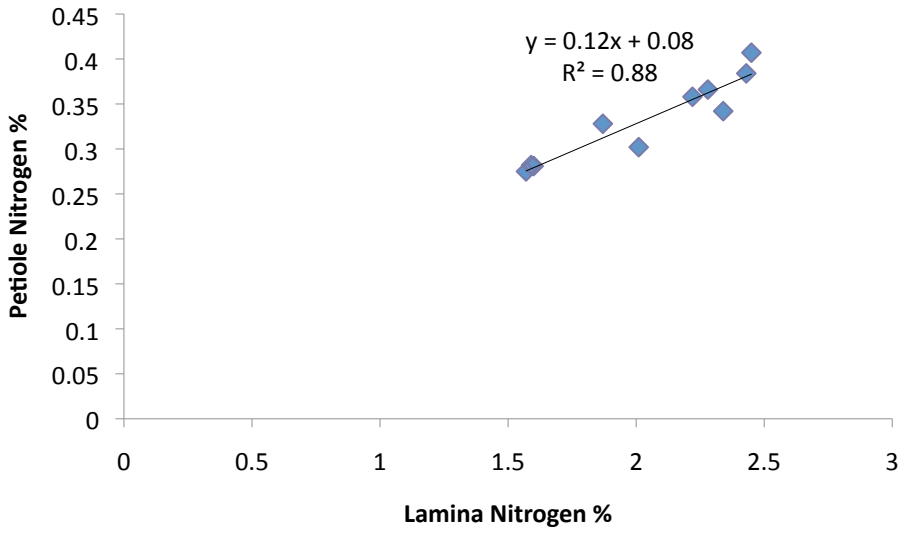

Figure 2.4-1 Lamina and petiole percent nitrogen comparison from Pinot noir samples taken on the 9th of January 2007.

A model was also developed to allow rapid and cost effective leaf nitrogen concentration assessment. This process involved two stages – initial analysis using a reference method and then NIR spectroscopy and chemometric analysis to develop the model.

Leaf lamina samples were collected from a range of The analysis for total nitrogen, carbon and hydrogen was determined by Dr Thomas Rodemann at the Central Science Laboratory, University of Tasmania, using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser. Between 0.7 and 1.7 mg of dried, finely ground sample produced using a cyclonic grinder were weighed into tin capsules using a Sartorius SE2 ultra-microbalance with an accuracy of 0.1 microgram. Combustion of the pressed tin cups was achieved in ultra high purity oxygen at 1000C using tungstic oxide on alumina as an oxidising agent followed by reduced copper wires as a reducing agent. The results were calibrated using a certified sulphanilamide standard. This analysis was done on leaves sampled at veraison in 2006-07, from the nitrogen rate by timing trial.

NIR reflectance spectra were also recorded by Dr Thomas Rodemann at the Central Science Laboratory, University of Tasmania, using a Bruker MPA with an autosampler wheel. The spectra were recorded using the integrating sphere between 12492 cm^{-1} and 3600cm^{-1} with a resolution of 4cm^{-1} using 64 scans. The Unscrambler 9.8 (CAMO Software) was used to generate the model using a partial least squares regression method.

Reference analyses were conducted using the method outlined above to verify the model on samples from 2007-08 (Figure 2.4-2).

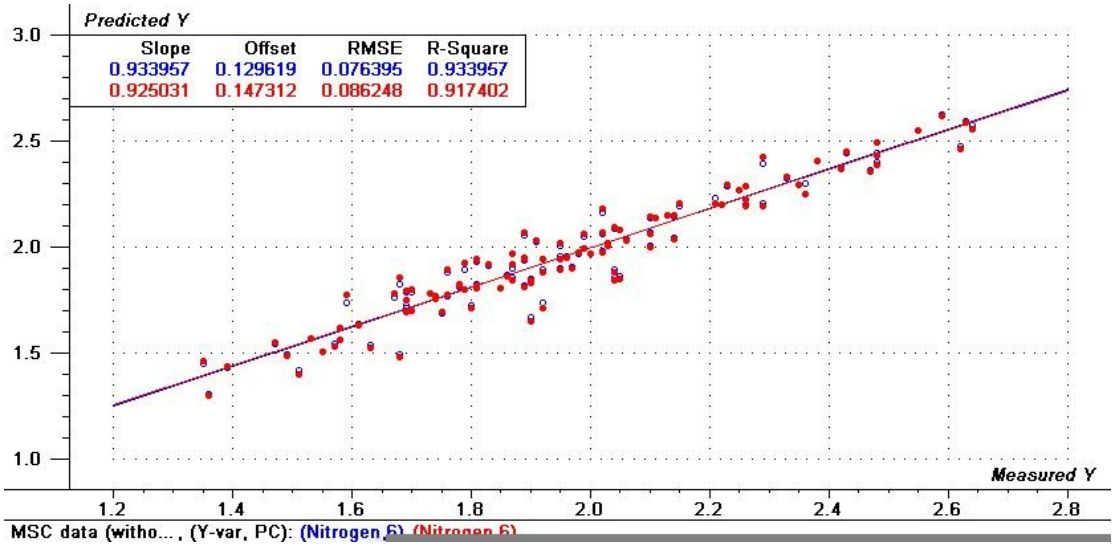

Figure 2.4-2 NIR model validation for total nitrogen concentration in grape leaf lamina, comparing predicted to measured nitrogen values

2.5 Fruit attribute measurements

Fallen berries and berry shrivel

Fallen berries were counted using a 400 mm x 600 mm rectangular quadrat. Before assessment the vine trunk was shaken three times with similar force used each time. The quadrat was placed on the ground under the vines, four times per measurement panel. The under vine row was divided into four sections, two per measurement vine, and the quadrat placed at random in each section. All fallen berries inside the quadrat were then counted.

The nitrogen addition by exposure trial was used to further examine the shrivelled berries. During processing of the fruit, berries were divided into two groups, one for shrivelled berries and another for non-shrivelled, or full, berries. All berries in each group were weighed together, and then subsets counted to get an average berry weight. This allowed a calculation of total berries per group.

Rachis measurements

Stems from the nitrogen addition by exposure trial were measured for total length from the point of the first lateral rachis branching (or scar if it was not present) to the base of the pedicels at the tip of the rachis (Figure 2.5-1).

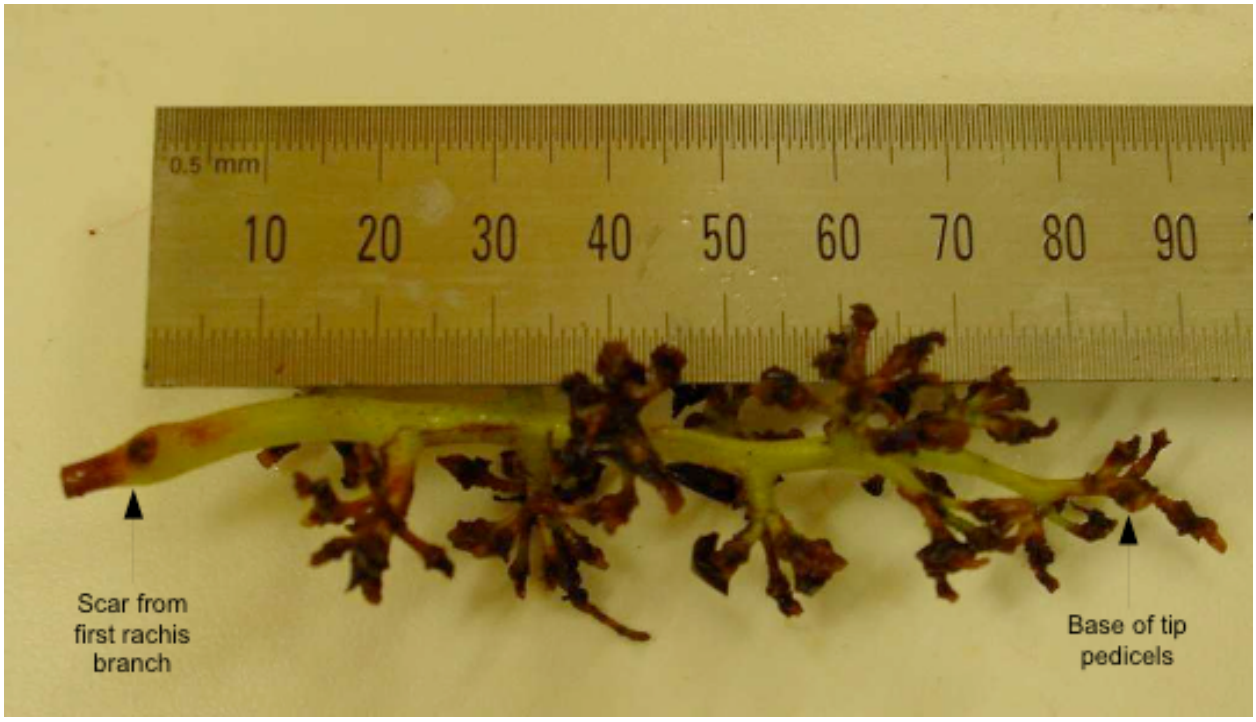

Figure 2.5-1 Measurement of rachis length

Figure 2.5-2 Counting rachis branches and pedicels of Pinot Noir bunches

The total number of rachis branchings was counted per rachis. Pedicels were then

counted on the 3rd, 6th and 9th rachis branching (Figure 2.5-2).

Averages were calculated for all these values. Branchlets per mm and pedicels per mm were also calculated.

Flowering date

Flowering progression was assessed in the 2006-07 season by a survey when the vines were judged to be around the middle of capfall.

The number of bunches on each monitor shoot were counted, and then the degree of capfall was assessed as a visual estimation of the percentage of flowers that had released their caps. To minimise any variability the same researcher carried out all assessment. Frequent "calibrations" were done, where the percentage of capfall was calculated.

Statistical analysis involved ANOVA of arcsine conversions of the percentages, to provide a normal distribution of data.

Flowering progression in the following season was estimated by grading shoots on the modified E-L scale developed in (Coombe, 1995). Modified E-L values between 20 and 26 are defined by the percentage of capfall.

Fruit set

In the 2007-08 season, fruit set was calculated by capturing and counting all flower caps. Fruit set assessments were carried out on vines that had formed the nitrogen by exposure trial the previous season, and received either no nitrogen or 100 g N/vine pre-bloom. Four bunches were chosen per panel at random, and marked with a plastic bread tag. Caps were captured in paper bags secured on the inflorescence by a plastic cable tie.

At the end of flowering, bags were removed. The caps were then cleaned to remove all anthers and other material. Any caps that were inadvertently retained in the non-cap material were counted to add to the total cap count later.

Cap counts were carried out by taking a random selection of caps, and two subsets of exactly 50 caps were separated from the main group. These were weighed, allowing calculation of an average cap weight, and then returned to the main group. The total weight of all the cleaned caps was then taken, allowing total caps to be calculated. This was added to the caps lost during cleaning.

The day before harvest of the main group of grapes, tagged bunches were removed. They had all berries removed, and rachis length, pedicels and branchlets counted. Fruit set was calculated by dividing pedicels by cap counts.

Fruit chemistry

Juice samples were taken from the grape must following crushing and destemming. Juice total soluble solids were assessed by a manual refractometer. In 2006 titratable acids (TA) were determined using the method of Iland et al. (2004), which involved a manual titration with NaOH to a pH of 8.2. A standard pH meter was used to find juice pH.

In 2007 and 2008, a Metrohm 785 DMP Titrino autotitrator (Metrohm Ltd, Herisau, Switzerland) was used to determine both pH and TA. TA was calculated to an end-point of pH 8.2, against 0.333M Na OH, in accordance with the autotitrator instructions.

Malate

Malate levels were determined enzymatically using a commercially available kit

manufactured by Vintessential Laboratories (Dromana, Australia). Spectroscopy was carried out on a Metertech SP8001 spectrophotometer (Taipei, Taiwan).

Yeast Assimilable Nitrogen

Yeast source nitrogen from the metabolisation of ammonium and free alpha amino acids. The nitrogen available from these two components are calculated separately then totalled to give the final yeast assimilable nitrogen (YAN). While some low molecular weight grape proteins may also be used as sources for nitrogen these only contribute a small amount of the total YAN, and therefore are not measured (Bell and Henschke, 2005).

Primary alpha amino acids were assessed using a kit purchased from Vintessential (Dromana, Australia), which follows the method of Dukes and Butzke (1998). Ammonium was also determined enzymatically, using a Vintessential kit. Both analyses were performed as detailed in the manufacturer's directions, using a Metertech SP8001 spectrophotometer.

Yield and yield components

Within each trial, all fruit was harvested on the same day. Fruit from the central two vines was harvested and weighed together. Bunches were counted during harvesting, and 100-berry samples were randomly selected and manually removed from a significant proportion of the harvested bunches and weighed to allow calculation of berry weight.

Berry extract analysis

Berry extraction and anthocyanin analysis followed the methods of the Australian Wine Research Institute recommended industry standard method, published by the Cooperative Research Centre for Viticulture. Analysis was performed on the 100-berry samples following weighing.

Frozen berry samples were homogenized in a Waring blender for 60 seconds on high speed. Approximately one gram of homogenate was then removed and transferred to a tarred 10mL plastic centrifuge tube. The total mass of homogenate was recorded to three decimal places.

10mL of 50% aqueous ethanol was added to the centrifuge tube, which was capped and mixed by inversion. Mixing was carried out by further intermittent inversion for at least one hour.

This tube was then centrifuged at 3500 rpm (RCN = 2333G) for 10 minutes in a Centurion EB series centrifuge (Centurion Scientific, West Sussex). The supernatant was then stored in the freezer until required.

The second stage of the analysis involved the transfer of 200 μL of extract to a plastic vessel containing 10 mL of 1.0 M HCl. This was incubated for 3 hours.

A Metertech SP8001 model spectrophotometer (Metertech Inc., Taipei) was zeroed using 1mL HCl in a quartz cuvette as a blank. 2 mL was transferred from the sample to a quartz cuvette with a 10mm path length. Absorbance was then read for each sample following transfer to at 250 nm, 280 nm, 300 nm and 520 nm.

Anthocyanin concentration as malvidin-3-glucoside equivalents was calculated using the formula:

Anthocyanins (mg/g homogenate) = (10.5 x A_{520} x DF x 10.5 x 1000)/(500 x 100 x HW)

Where:

A_{520} is the absorbance at 520 nm

DF is the dilution factor from adding the extract to the HCl (11 in this case)

10.5 is the total extract volume estimate (based on a homogenate weight between 0.95 and 1.05 g)

HW is the homogenate weight in grams

Phenolics were calculated using a model developed by R. Dambergs of the Australian Wine Research Institute (Unpublished). The model had been developed comparing wine spectral signatures against methyl cellulose precipitatable tannins (Sarneckis et al., 2006). The spectral signatures were analysed to determine which wavebands were best able to indicate the different tannin levels. The model used in these trials was based on absorbances at 250, 280 and 300 nm. These were assessed using quartz cuvettes with a 10 mm pathlength,

2.6 Winemaking methods

Winemaking – 2006

Winemkaing in 2006 was carried out on grapes from the nitrogen by irrigation trial, and block A and block B Pinot Noir vigour trials. Fruit for the nitrogen by irrigation trial came from the two central measurement vines in each treated panel. Fruit in the vigour trials came from all four vines in a panel. Grapes were destemmed and transferred to a fermentation vessel (either a plastic 20 L buckets or 20 L stainless steel bucket), and potassium metabisulphite added to provide a free sulphur level of 60 ppm. Enzymes were added (Lafase HE, Laffort), prepared according to the manufacturer's recommendations.

Musts were allowed to warm up to at least 16 degrees prior to inoculation. Ferments were inoculated with 300 ppm RC212 yeast (Lalvin), prepared according to the manufacturer's recommendations, including an acclimation step.

Cap management in 2006 used a punch-down system, with fermenters plunged twice a day using a potato masher and carrying out ten plunges at each session. Following the punching down, ferment temperature was recorded and density assessed by hydrometer. Fermentation was carried out in a heated insulated room, with fans being used to ensure temperature was constant throughout. Temperature was maintained at around 25°C, and were covered while not being plunged.

Fermentation proceeded until all ferments had a Baumé below 1, and then pressed. The press was a small basket press, with the pressure to be applied by hand. Wines were pressed into either two or three litre PET bottles (Caled Containers, Tasmania Australia), with dry ice (solid CO_2) used to protect the wine against oxygen. The bottles were not completely filled to maintain a generous headspace and the lid was loosely sealed to allow further CO_2 release. They were returned to the warm room to complete primary ferment. After fermentation had ceased, wines were settled for around three days and then racked off solids.

They were then inoculated with a standard commercial malolactic bacteria (Chr. Hansen, Denmark), and were monitored for completion of malate breakdown using thin layer

chromatography (kit available from Vintessential Laboratories, Victoria, Australia) and enzymatically with a spectrophotometer using the method outlined in the juice analysis section of this materials and methods section. Oxygen was prevented from contacting the wine by using the flexible nature of the PET bottles to squeeze gas out, before sealing the lid. This effectively reduced the headspace to a minimum.

On completion of malolactic fermentation wines were racked off lees into a fresh PET vessel, again under protection from CO_2. Potassium metabisulphite was added at a rate to supply free SO_2 levels of 57 ppm. Wines were left to settle in a cool room running at around 4°C.

After settling for one month, wines were racked and bottled into 750mL glass bottles with screw caps. Sulphur was adjusted following racking to a level of 30ppm of free SO_2, as measured by the aeration-oxidation method (Iland, 2004). All wine movements were conducted under dry ice protection, and by positive pressure by nitrogen gas. Sulphur was checked again in bottled wines and found to be low after about seven months in bottle. A number were checked, and on the basis of their results a blanket addition of 20ppm of sulphur was added. CO_2 was used to ensure that there was minimal oxygen contact.

Winemaking – 2007 and 2008

Winemaking was carried out in 2006-07 on the nitrogen timing by rate trial, the irrigation by nitrogen trial, and the nitrogen by bunch exposure trial in the nitrogen application studies, and block A Pinot Noir in the vigour trials. In 2008, wines were made from the nitrogen timing by rate trial the field nitrogen and winery nitrogen comparative study in the nitrogen application studies, and block A Pinot Noir in the vigour trials.

In all nitrogen application rate and timing trials, fruit was harvested from the central two vines in the panel and the weight recorded. Where required, fruit from the buffer vines in the same panel was then harvested to bring the total fruit weight up to 12 kg. Where fruit from the two vines was greater than 12 kg fruit was removed.

In the vigour gradient trials, and field nitrogen versus winery nitrogen trials all vines were harvested. After weighing the total yield, excess fruit was removed to leave 12 kg of fruit for fermentation

Crushing of grapes was carried out using a Baesso 80kg crusher/destemmer unit (Australian Winemakers), following chilling in a 2°C cool room. Fruit was crushed directly into the fermentation vessel, and potassium metabisulphite added at equivalent rates to the previous season. Fruit was taken to a 25°C room for warming prior to inoculation, and enzymes were added as per the previous season.

Yeast preparation and addition was unchanged from 2006.

Fermentation was carried out at around 25°-26°C. Variance in temperature between fermenters was less than 2°C at any measurement period.

There were two cap management systems used in 2007 and 2008. Punch-down cap management involved twice-daily plunging sessions to break up the cap, with ten plunges using a potato masher at each session. Submerged cap management systems used a heading down board to hold the grape skins under the liquid, preventing the cap from ever becoming dry and reducing labour. Refer to p. 178 for a comparison of the two techniques.

Fermentation monitoring was carried out using an Anton Paar portable density meter DMA 35N (Anton Paar GmbH, Graz, Germany). This measured both Baumé and

temperature of the ferment, with minimal liquid required.

Pressing was carried out using a 20L Idro brand water bag press (Australian Winemakers) to a maximum pressure of 2 bar (200kPa). Pressure was maintained until minimal liquid continued to flow. Wines were pressed off skins at between -0.5 and 0.8 units Baumé in 2007 and at below 0 Baumé in 2008.

Wines were pressed into 3L PET containers (Caled Containers, Tasmania Australia) under dry ice protection. They were settled to remove gross lees and then racked again. The fruit volumes used allowed two 3 L PET bottles to be nearly filled; any excess was discarded.

In 2007 malolactic acid bacteria were added to wines made from fruit harvested from the vigour gradient trial. Malolactic fermentation was carried out in the PET vessels, and monitored for malate levels by a malate enzymatic analysis kit produced by Vintessential (Dromana, Australia). On completion of malolactic fermentation, wines were racked into a blending vessel, and then into clean PET containers, with the addition of 57ppm SO_2 from a 10% stock solution. They were then stored at 2°C until bottling.

Following pressing, all other wines were monitored for primary ferment progression using Clinitest tablets (Ames Division, Miles Laboratories, Illinois), until there was less than 0.25 g/L sugars remaining. They were then chilled by storing at 2°C and when settled the clear wine was racked off the solids, blending all PET bottles in each treatment into a single blending vessel. Potassium metabisulphite was added at a rate of 57 ppm free SO_2. They were returned to clean 3 L PET bottles and left at 2°C until bottling. In 2007 bottling commenced 15 months after racking.

During bottling, wines were racked, re-blended and had free SO2 levels adjusted with potassium metabisulphite addition. Sulphur levels were tested using aeration-oxidation (Iland, 2004) and adjusted to around 30ppm free SO_2.

Final filtration and bottling took place in one movement. Wines were filtered through two membrane cartridge filters in series (Tenco, Italy), the first with a pore size of 1µm, and the second with a pore size of 0.25µm. They were bottled into screw cap 750 mL wine bottles and also smaller 100 mL screw cap sample bottles for immediate colour testing. All movements were carried out under inert gas protection.

2.7 Wine assessment

Somers analysis

Wines were analysed by the modified Somers analysis (Mercurio *et al*, 2007). This involved assessment of The colour density (with and without sulphur dioxide correction), hue, anthocyanin concentration, anthocyanin ionisation, chemical age1 and chemical age 2, pigmented polymers, and phenolics were calculated using Somers calculations (Somers and Evans, 1977). Sulphur resistant pigments were calculated using the method described in Mercurio et al (2007).

Tannin analysis

Tannin concentration was assessed using a method developed at the Australian Wine Research Institute (AWRI) (R. Dambergs, pers. comm.). Wines were centrifuged for 10 minutes at 3500rpm (RCN = 2333G) in a Centurion Scientific EB series centrifuge before analysis. The supernatant wine was then diluted adding one part of wine to 50 parts of 1M aqueous hydrochloric acid (51 x dilution factor). After incubation at room

temperature for four hours, the absorbance at 250 nm, 270 nm, 280 nm and 300 nm was measured on a Metertech SP8001 spectrophotometer, using quartz cuvettes with a 10 mm path length. A spreadsheet was used containing a chemometric tannin determination calculation. The model was developed using analysis of spectral characteristics of wines with reference to their tannin concentration assessed using the methyl cellulose precipitate assay (Sarneckis et al., 2006).

MS e_nose analysis

Mass spectrometry (MS) e_nose analysis was carried out at the Australian Wine Research Insitute, and PCA analysis carried out on the spectra of each wine using *The Unscrambler* software package (version 9.1, CAMO ASA, Norway), following the method published by Cozzolino et al. (2005). MS e_nose analysis involves headspace sampling but unlike gas chromatography-MS (GC-MS) there is no chromatographic separation before MS analysis. Combined with chemometrics this technique allows differences between samples to be analysed, and is not susceptible to interference by retention time shift due to chromatography, which can cause problems with GC-MS (Cozzolino et al., 2009). To avoid ethanol effects, the mass profiles relating to ethanol were not included in the spectra.

2.8 Statistical analysis

ANOVA analyses were performed using Genstat statistical software (VSNi, UK). Correlation tables were established in Excel, generating Pearson's correlations and R^2 values. Significance of regressions was assessed using Genstat.

3 Impact of vine vigour on leaf health, canopy growth, fruit attributes and wine quality in *Vitis vinifera* L. cvs. Pinot Noir and Sauvignon Blanc

3.1 Introduction

Vine shoot vigour may lead to changes in vine leaf health and wine quality. The following trials selected areas within vineyard blocks where vigour changes occurred in a small area in blocks of Pinot Noir and Sauvignon Blanc.

The first chapter in this section details assessments of links between leaf health and overall canopy growth, using a range of methods including ground-based assessments and remote sensing using near infrared imaging.

The second chapter investigates how the changes in vine vigour and leaf health were impacting fruit yields and fruit chemical attributes.

The final chapter aimed to link the vine vigour to wine quality, by investigating changes in fermentation dynamics and wine attributes, including sensory assessments.

By undertaking a review of the winemaking process from vineyard to bottling, links may be made between vigour and leaf health, and the resulting wine. The results of these trials may assist in understanding of how vigour affects wine quality in a cool climate, and what management steps may be applicable to maximise quality in heterogeneous vineyards.

3.2 Investigations into vine vigour and links to leaf health in grapevine (*Vitis vinifera* L.) cvs Pinot Noir and Sauvignon Blanc

3.2a Introduction

A vine's capacity for growth refers to the total amount of growth that a vine can support, while vine vigour relates to the rate of shoot growth (Dry and Loveys, 1998, Winkler, 1970, Keller, 2010). Vines grown in high capacity sites will tend to increased numbers of high vigour shoots, which has many implications for wine quality (Smart, 1985).

A site's capacity for growth varies due to a number of dynamic and static factors. Dynamic factors include soil and air temperature, moisture and nutrient availability (Keller, 2004, Winkler, 1970, Ussahatanonta et al., 1996). Soil qualities such as pH, texture and depth are static factors (Fiorillo et al., 2009) that will also influence the capacity for growth. Few soils vineyards are completely homogeneous, leading to differences in capacity for growth within one management unit being very common (Bramley and Hamilton, 2004, Proffitt et al., 2006).

Shoot vigour is linked to leaf health. Plant hormones such as abscisic acid (ABA) rise with stress (Dry and Loveys, 1998, Hartung et al., 2005, Jiang and Hartung, 2007, Wasilewska et al., 2008), leading to decreased shoot tip activity and activating senescence processes, while cytokinin hormones promote tip growth and delay senescence (Gan and Amasino, 1997). Cytokinin production increases when there is a high nutrient availability and is reduced when the vine faces nutrient deficiency or water stress (Sakakibara, 2006). Therefore, conditions that promote vigorous shoot growth and increased leaf numbers will also lead to reduced leaf senescence and raise leaf chlorophyll, while conditions that reduce shoot growth may accelerate the onset and progression of senescence and reduce leaf chlorophyll concentration and photosynthesis. Conversely, shading may also lead to senescence (Gan and Amasino, 1997), and vines with dense canopies are observed to have senescing leaves in the canopy interior (Smart, 1985).

Increasing canopy vigour leads to longer shoots with more laterals and larger leaves (Smart, 1985), which can alter the vine canopy microclimate, by progressively increasing shading and reducing air movement within the canopy. Canopy density is altered by changes in leaf health, which may lead to increasing leaf senescence due to shading or stress, decreasing density. Dense canopies can impact product quality by leading to higher disease levels (English et al., 1989), lower fruit set (Dry, 2000) and reduced phenolic concentration (Cortell and Kennedy, 2006, Cortell et al., 2008). Conversely, canopies with insufficient vigour may have excessive fruit exposure, which can cause damage from increased berry temperature and radiation, as well as reduced yields (Dry, 2000).

Canopy growth can be assessed in a number of ways. Pruning weights allow assessment of overall vine growth and are correlated to leaf area, while point quadrat analysis is a direct measure of canopy density. Visual scorecards can also be a convenient way to judge canopies (Smart and Robinson, 1991). Recent developments in remote sensing using near infrared (NIR) imaging allow rapid assessments of differences in vine canopy growth across large areas (Bramley and Hamilton, 2004, Proffitt et al., 2006, Dobrowski et al., 2002, Johnson et al., 2003, Lamb et al., 2004, Stamatiadis et al., 2006). This technique uses a comparison of reflected NIR light with reflected red light to generate one of several indices which correspond with the amount of photosynthetically active

biomass in the area of interest (Hall et al., 2002). Two indices in particular have been used in viticulture, the Plant Cell Density (PCD) and the Normalised Differential Vegetation Index (NDVI). Both NDVI and PCD have been correlated to pruning weights (Johnson et al., 1996, Carnevali et al., 2009), leaf area (Johnson et al., 2003, Carnevali et al., 2009), canopy density (Hall et al., 2008, Dobrowski et al., 2002) and canopy area (Hall et al., 2008) of grapevines.

This study investigates the links between a range of commonly used canopy assessment methods, with the aim of clarifying how each is linked both to each other.

Hall et al. (2002) identified a number of requirements to achieve integration of remote sensing technology with viticultural production. These include the development of research to link remote sensing output with yield and quality parameters. Further information towards such integration was one objective of the trials described in this chapter.

A particular aim of this study is to investigate how late season leaf health is linked to other canopy attributes. Late season leaf health may be useful as a monitoring tool because relative differences can be rapidly visually assessed. The leaf and canopy information described in this chapter form part of a larger trial investigating links between canopy attributes, in particular leaf health, and wine composition and quality.

3.2b Materials and Methods

Trial vineyard description

Four trial sites were used in the 2005-06 season. Two were Pinot Noir blocks (block A and block B), and two were in Sauvignon Blanc (block C VSP (vertical shoot positioned trellis) and block C SH (Scott Henry trellis)).

Block A Pinot Noir trials were continued in 2006-07 and 2007-08, using the same vines as in 2005-06. Trials were also conducted on Scott Henry trellised vines in the Sauvignon Blanc block (block C-07), although these trials were not on the same vines used in 2005-06.

For further details about site layout, please refer to the general materials and methods section (p. 17).

Table 3.2-1 summarises analyses undertaken in these trials, including references to the general materials and methods section as appropriate.

*Table 3.2-1 Vigour trial analysis summary for vegetative growth and leaf health measurements. Reference pages refer to location within the General Materials and Methods section, for more information on detailed methodology. * - more information on this analysis below the table*

Trial	*Analysis*	*Date*	*Reference page*
Block A Pinot Noir	Aerial vigour map	02/06 02/07	25
	Vigour scorecard	7/04/06	24
	Leaf retention*	8/05/06	
	Bud burst %*	31/05/07	
	Leaf chlorophyll estimate	20/03/07	26
	Chlorophyll fluorescence	20/03/07	26
	Point quadrat	19/03/07	28
	Periderm development*	26/02/07	29
	Pruning weight	25/05/06 31/05/07	28
Block B Pinot Noir	Aerial vigour map	02/06	25
	Pruning weight	25/05/06	28
	Bud burst %*	28/09/07	
Block C VSP	Aerial vigour map	02/06	25
	Leaf retention*	10/05/06	
	Pruning weight	7/07/06	28
Block C SH	Aerial vigour map	02/06	25
	Pruning weight	7/07/06	28
Block C 07	Aerial vigour map	02/07	25
	Leaf chlorophyll estimate	19/03/07	26
	Chlorophyll fluorescence	19/03/07	26
	Point quadrat	19/03/07	28
	Leaf nutrient analysis	27/01/07	29

Leaf chlorophyll concentration

Samples were taken from 18 randomly selected leaves in the fruiting zone and a further 18 randomly selected leaves were sampled in the upper part of the canopy, all from upwards-pointing shoots originating from the upper cane arms. Each leaf was measured at two different points and this was then averaged to give a final result.

Point quadrat

Point quadrat measurements were taken at each canopy level with 30 insertions were made along the length of the panel.

Leaf retention

Leaf counts were carried out on the 8th of May 2006, to estimate late season leaf retention. In block A Pinot Noir, between 6 and 9 upward facing shoots (shoots from upper arms) and 3 to 5 downward facing shoots (shoots from lower arms) were randomly selected. In block C-VSP, between 8-10 shoots were selected, all upwards facing since the trellis was a VSP. Each shoot had all leaves present counted, either as main shoot leaves or as lateral leaves. Main shoot and lateral nodes were also counted. Leaf retention was calculated as the percentage of nodes still with a leaf.

Periderm formation

Periderm development in vigour zones 1, 2 and 4 in block A Pinot Noir were assessed at veraison in 2006-07, with zone 3 missed due to time constraints.

Bud burst

Bud burst in block A Pinot Noir in the 2006-07 season was determined during pruning weight assessment, when canes per vine and nodes per vine were counted. The number of canes was divided by the number of nodes laid down at the previous pruning to give an estimate of the number of buds that had burst, expressed as a percentage.

Bud burst in block B Pinot Noir was assessed in the spring of the 2006-07 season. Nodes and shoot numbers were counted. As in block A, the node number divided by the shoot number was used to calculate bud burst percentage.

Statistical analysis

ANOVA analyses were performed using Genstat statistical software (VSNi, UK). Correlation tables were established in Excel, generating Pearson's correlations and R^2 values. Significance of regressions was assessed using Genstat.

3.2c Results

Plant cell density (PCD) increased with vigour between all vigour zones in both 2005-06 and 2006-07 in block A Pinot Noir (Table 3.2-2). PCD values in 2005-06 were also significantly different for all but the lowest two vigour zones in block B Pinot Noir (Table 3.2-2), and for high and low vigour zones in block C Sauvignon Blanc (Table 3.2-3).

*Table 3.2-2 Plant Cell Density (PCD) values for 2005-06 and 2006-07 across four vigour zones in block A Pinot Noir, and across five vigour zones in block B Pinot Noir at veraison. PCD is scaled to an 8-bit data range (0-255). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Block A (Pinot Noir)		***Block B (Pinot Noir)***
Vigour levels	2005-06	2006-07	2005-06
1	88a	115a	63a
2	114b	143b	62a
3	138c	167c	118b
4	179d	211d	135c
5			173d
Sig	***	***	***

*Table 3.2-3 Veraison PCD values from the Sauvignon Blanc vigour trial sites in block C-SH and block C-VSP in 2005-06. PCD is scaled to an 8-bit data range (0-255). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	***Block C-VSP***	***Block C-SH***
Low	83a	78a
High	164b	174b
Sig	***	***

Scorecard results

Leaf size and leaf colour in 2005-06 in block A Pinot Noir (Table 3.2-4) both increased in response to increasing vigour, although no significant difference was detected between the two lowest vigour zones. Canopy attributes (gaps, density, fruit exposure and leaf loss) all increased as vigour level increased. The total score from the vigour scorecard was significantly different in all vigour zones.

*Table 3.2-4 Vigour scorecard from block A Pinot Noir at veraison in the 2005-06 season. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	***Leaf size***	***Leaf colour***	***Canopy gaps***	***Canopy density***	***Fruit exposure***	***Average leaf loss***	***Total Score***
1	2.3a	2.1a	1.4a	1.2a	1.0a	2.4a	10.3a
2	2.5a	2.4a	2.3b	1.4a	2.1b	3.3b	13.9b
3	3.5b	3.3b	3.4c	2.4b	3.1c	3.8bc	19.5c
4	4.0b	3.7c	4.0c	3.2c	4.8d	4.4c	24.1d
Sig	***	***	***	***	***	***	***

Bud burst

Bud burst percentage was reduced on lower vigour vines in block A in 2006-07 (Table 3.2-5). This was offset by an increase in node number on the arms of low vigour vines. As a result, there was no difference in the number of shoots per cane.

Block B Pinot Noir in 2006-07 showed a different pattern of bud burst (Table 3.2-6). While percent bud burst also rose with increasing vigour in this trial, the buds per cane did not alter. Therefore, shoots per cane also increased with increasing vigour.

*Table 3.2-5 Bud burst percentage, buds per cane and shoots per cane across different vigour zones in 2007 in block A. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Bud burst percentage	Nodes per cane	Shoots per cane
1	51a	9.4b	4.8
2	58ab	9.2b	5.4
3	63b	8.5ab	5.3
4	73c	7.7a	5.2
Sig	**	**	n.s.

*Table 3.2-6 Bud burst percentage, buds per cane and shoots per cane across different vigour zones in 2007 in block B. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Bud burst percentage	Nodes per cane	Shoots per cane
1	66ab	10.2	6.7a
2	65a	9.8	6.5a
3	65a	10.3	6.9a
4	74bc	9.4	7.4ab
5	82c	10.1	9.0b
Sig	***	n.s.	*

Pruning weights

Increased vigour in block A was associated with increased pruning weights and mean cane weights in 2005-06 and 2006-07 (Figure 3.2-1). The consistency between seasons clearly indicated the stability of the growth patterns in the field.

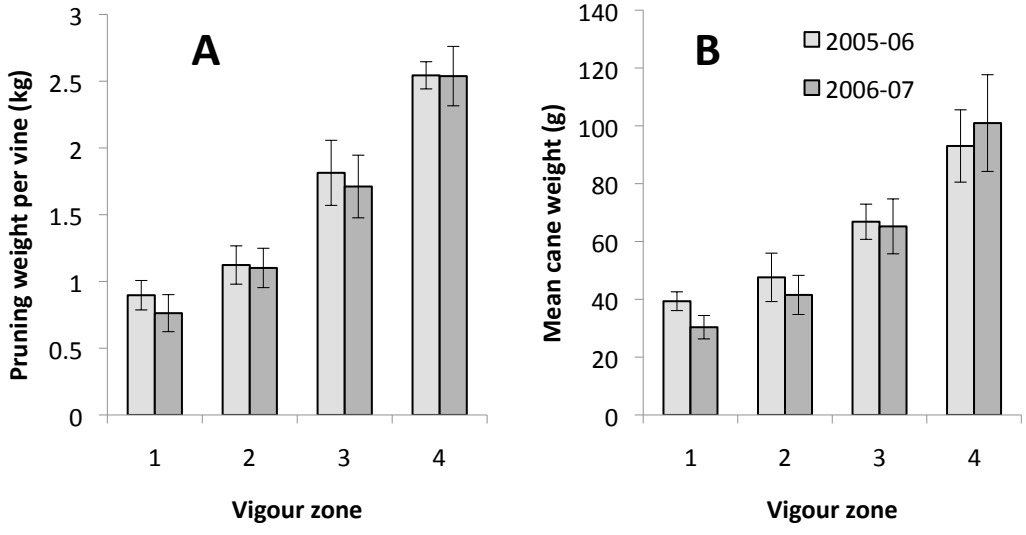

Figure 3.2-1 Block A pruning weights (A) and mean cane weights (B) in 2005-06 and 2006-07. Error bars represent standard errors of the mean.

Pruning weights in blocks B, C-VSP and C-SH also increased as the vigour level increased (Figure 3.2-2, Table 3.2-7).

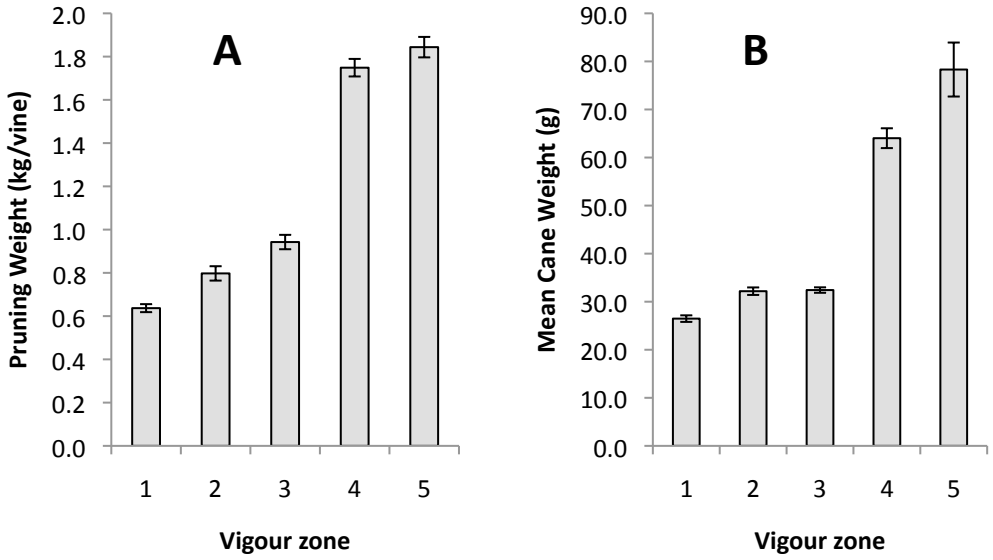

Figure 3.2-2 Pruning weight and mean cane weight in block B Pinot Noir in 2006. Error bars represent standard errors of the mean

*Table 3.2-7 Pruning weights, cane weights and shoot counts for Sauvignon Blanc trial sites in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Pruning weight (kg/vine)		***Mean cane weight (g)***	
Vigour	Block C-VSP	Block C-SH	Block C-VSP	Block C-SH
Low	0.76a	1.02a	36a	41a
High	1.78b	2.47b	109b	79b
Sig	***	***	***	***

Late season leaf retention

Increasing vigour was associated with greater retention of leaves in block A when assessed on the 8th of May 2006 (Table 3.2-8). This was as a result of more leaves on the main shoot and on laterals being retained, as well as an increase in the number of lateral nodes. There was no significant difference between the two lower vigour zones in any of the leaf or node counts. There was an increase in leaves retained on lateral shoots compared to main shoots.

*Table 3.2-8 Late season leaf retention assessed on the 8th of May 2006 season in block A (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	***Main shoot leaves total***	***Main shoot nodes***	***Percent retention***	***Lateral leaves total***	***Lateral nodes***	***Leaves per node***
1	0.11a	13.9	1a	0.02a	1.74a	1a
2	0.89ab	14.3	7ab	0.23a	1.89a	5a
3	1.72bc	13.8	15b	0.97a	5.25b	21b
4	2.02c	13.4	19b	5.10b	13.46c	41c
Sig	**	n.s.	**	***	***	***

Leaf retention was also increased in block C-VSP on the 8th of May, both in total leaf counts and also as a percentage of nodes with leaves still retained (Table 3.2-9).

*Table 3.2-9 Late season leaf counts per shoot and leaf retention in block C-VSP in 2005-06, on main shoots and lateral shoots. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Main shoot leaves*	*Leaf retention %*	*Lateral shoot leaves*	*Leaf retention %*
Low	1.2a	5.8	0.6	26.2
High	2.7	14.5	21.5	51.3
Sig	**	***	***	***

Leaf Chlorophyll Concentration and Chlorophyll Fluorescence

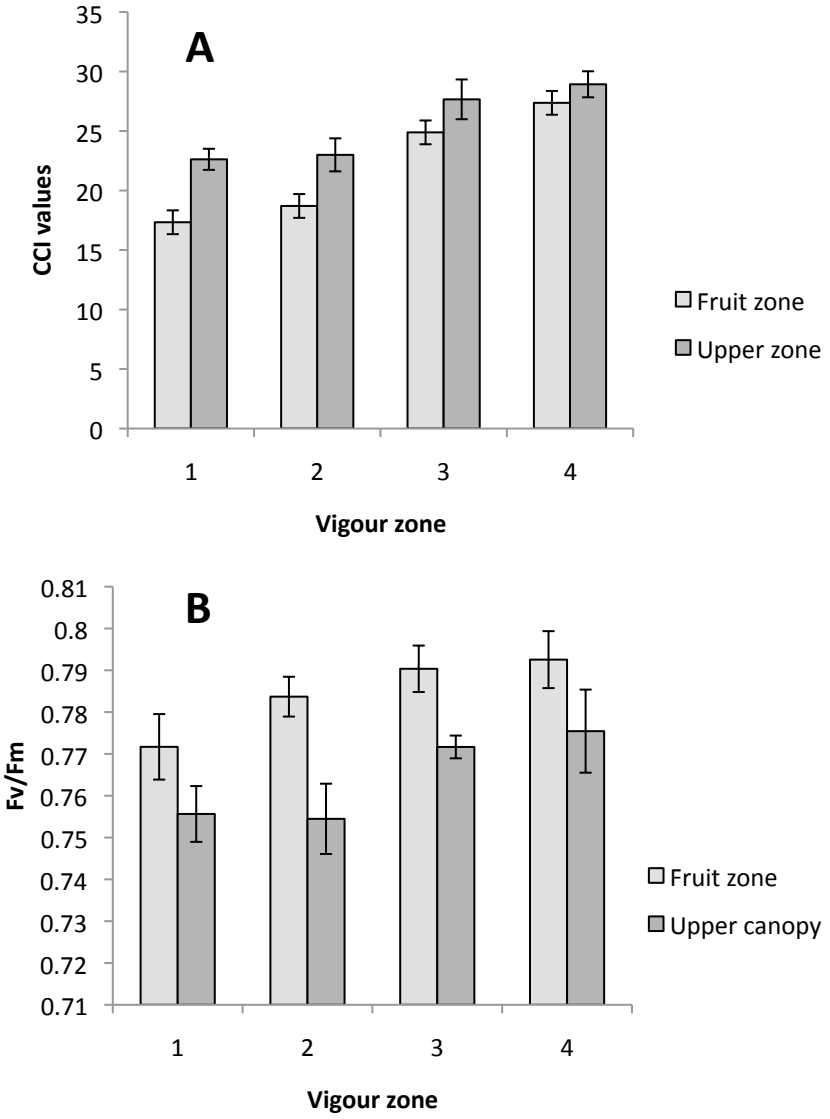

Figure 3.2-3 Leaf chlorophyll estimates as CCI values (A) and F_v/F_m values (B) from block A on the 20th March, 2007. Values for the fruiting zone and the upper zone are given separately. Error bars represent standard errors of the mean.

Leaf chlorophyll estimations increased as vigour zone increased in both block A (Figure 3.2-3) and in the Sauvignon Blanc trial (Table 3.2-10) in 2006-07. While there were no significant differences in leaf chlorophyll or photosynthetic efficiency in block A

between the two lower vigour zones, chlorophyll levels were higher in vigour zones 3 and 4. The leaves in the upper canopy had higher average chlorophyll concentration than those in the fruiting zone.

The chlorophyll fluorescence measurements from block A (Figure 3.2-3 B) and block C-07 (Table 3.2-10) indicate that there is a greater degree of stress on vines in the low vigour zones, which is decreasing photosynthetic efficiency. Photosynthetic efficiency was lower on leaves higher in the canopy.

Chlorophyll concentration was significantly higher in the high vigour zone in the Sauvignon Blanc, along with an increase in photosynthetic efficiency.

*Table 3.2-10 Chlorophyll fluorescence parameters and chlorophyll concentration index in Sauvignon Blanc block C-07 on the 19th of March, 2007. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	*F_v/F_m*	*CCI*
Low	0.73a	8.3a
High	0.79b	19.7b
Sig	**	***

Point Quadrat

Point quadrat analysis revealed a number of canopy differences between vigour zones in block A. Combining all the data from both the upper and fruiting zones showed a clear increase in internal leaves and total leaf layer numbers, except between vigour levels 3 and 4 (Table 3.2-11).

Significant differences existed between the different canopy zones for a number of the point quadrat measures. Leaf layer number continued to increase in the upper canopy zone between vigour zones 3 and 4, whereas there was a noticeable plateau in the fruiting zone, with no significant differences between vigour zones 2, 3 or 4 (Table 3.2-12).

Yellow leaf counts were also significantly different between the zones (Table 3.2-12). The fruiting zone yellow leaf counts climbed in a regular fashion, as vigour increased. Contrasting this, upper leaf counts showed no sign of senescence in the lower vigour zones, but a rapid climb as vigour increased.

*Table 3.2-11 Point quadrat data, averaged across both the fruiting and the upper canopy zones, for block A Pinot Noir on the 20th March, 2007. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	*Internal leaves*	*Leaf layer number*	*Yellow leaves*	*Gaps*
1	0.21a	1.41a	0.03a	0.24b
2	0.43b	1.95b	0.10a	0.10a
3	0.71c	2.31c	0.20b	0.08a
4	0.84c	2.53c	0.42c	0.03a
Sig	***	***	***	***

*Table 3.2-12 Point quadrat data differences between fruiting zone and upper canopy zone (data with no significance between zones not shown) for block A Pinot Noir on the 20th March, 2007. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	Leaf layer number fruit zone	Leaf layer number upper zone	Yellow leaves fruit zone	Yellow leaves upper zone	Canopy gaps fruit zone	Canopy gaps upper zone
1	1.7b	1.2a	0.05a	0.0a	0.16c	0.30d
2	2.3cd	1.9bc	0.18b	0.01a	0.05ab	0.14c
3	2.6d	2.5d	0.25b	0.16b	0.05ab	0.10bc
4	2.6d	3.4e	0.37c	0.48c	0.06ab	0.01a
Sig	***		**		**	

Leaf layer number, internal leaves and percent gaps were also significantly greater in the upper canopy zone of high vigour Sauvignon Blanc vines in block C in 2006-07, however there were no significant differences in these attributes in the fruit zone (Table 3.2-13, Table 3.2-14). Yellow leaf counts did not change significantly in response to vigour either in the fruit zone or the upper canopy zone. There were a greater proportion of clusters that were internal, as well as a greater number of total clusters, and a higher cluster exposure layer in the higher vigour zones.

*Table 3.2-13 Point quadrat results from Sauvignon Blanc (block C-07) on the 19th of March, 2007 in the fruit zone. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Leaf Layer Number	Internal leaves	Yellow leaves	Percent gaps	Leaf exposure layers	Occlusion layers	Internal clusters	Total clusters	Cluster Exposure layer
Low	1.4	0.22	0.21	13.5	0.24	1.7	0.08	0.25	0.09
High	1.8	0.30	0.18	5.0	0.30	2.2	0.22	0.48	0.23
Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*

*Table 3.2-14 Point quadrat results from the upper canopy region in the Sauvignon Blanc trial site on the 19th of March, 2007. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Leaf Layer Number	Internal leaves	Yellow leaves	Percent gaps	Leaf exposure layers
Low	1.2a	0.15a	0.11	21b	0.19a
High	2.1b	0.53b	0.11	7a	0.45b
Sig.	**	**	n.s.	*	*

Periderm development

Periderm development increased in the highest vigour zone vigour, with a greater number of nodes lignified. There was no significant difference between the two lower vigour zones (Figure 3.2-4).

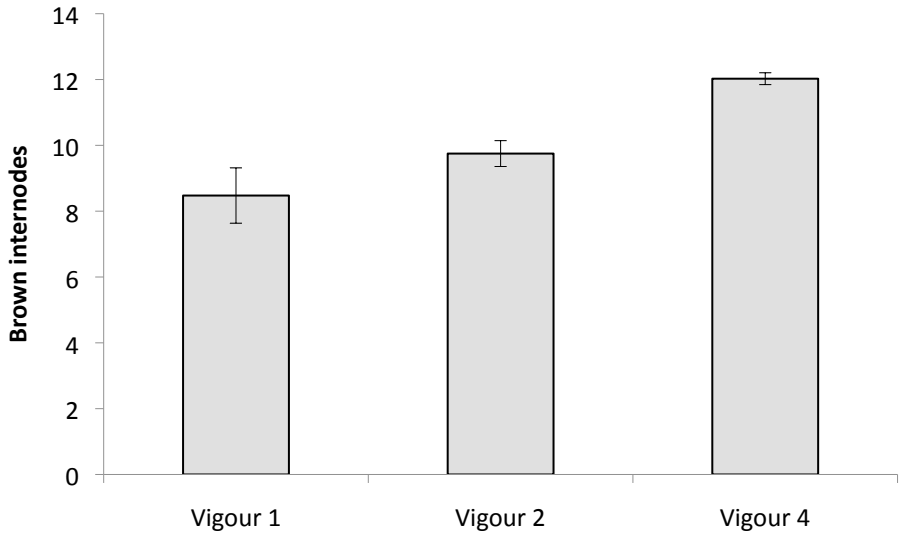

Figure 3.2-4 Impact of vine vigour on the number of internodes with periderm browning in zones 1,2 and 4 in block A, at veraison 2006-07. Error bars represent standard errors of the mean.

Leaf nutrient analysis

Block C -07 Sauvignon Blanc vines showed a number of differences in leaf nutritional status (Table 3.2-15).

*Table 3.2-15 Veraison leaf lamina nutrient analysis from Sauvignon blanc block C-07. Suitability guidelines are from Robinson (1992). D=Deficient; A=Adequate; E=Excessive; Two levels indicates that the different zones vary in their suitability. No suitability level indicates that standards were not given in the literature. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Total N %*	*Phosphorus %*	*Potassium %*	*Sulphur %*	*Sodium %*	*Calcium %*	*Magnesium %*	*Copper mg/kg*	*Zinc mg/kg*	*Manganese mg/kg*	*Iron mg/kg*	*Boron mg/kg*
Low	1.7a	0.25	1.5	0.26	0.04	2.0	0.48	266	34	148	73	51
High	2.4b	0.18	1.5	0.31	0.03	1.9	0.39	239	34	294	100	52
Sig	***	**	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	**	*	n.s.
Suitability	A	A	A	-	D	A	A	A	A	A/E	-	A

Correlation studies

The panel PCD index average showed a strong positive correlation with pruning weight (Figure 3.2-5).

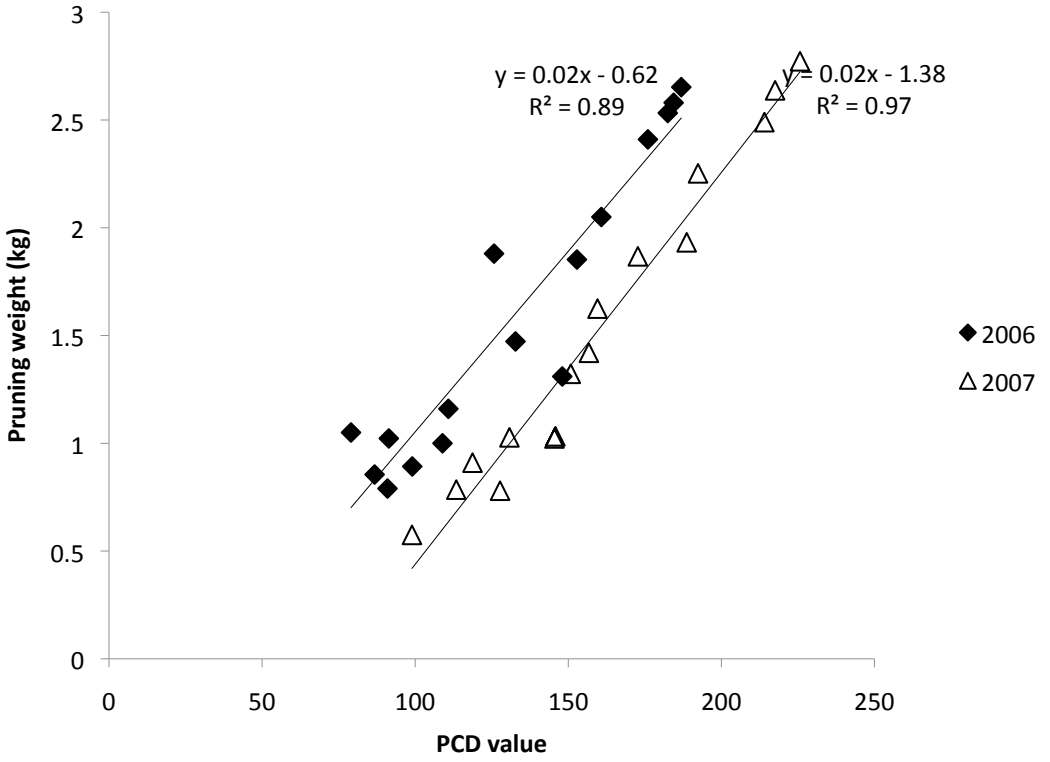

Figure 3.2-5 Plant cell density (PCD) assessment (converted to greyscale 8-bit values) against pruning weight across two years in block A

The 8 bit PCD values were assessed for all trial blocks in the 2005-06 season, and compared to pruning weights. Regression studies were applied to all vines trellised to a Scott Henry trellis, and indicated that all vines trellised similarly had a similar correlation between pruning weight and PCD (Figure 3.2-6). This included both Pinot Noir (blocks A and B) and Sauvignon Blanc (block C-SH). Note that when forced through zero, the linear regression equations both had an equal slope (y=0.012x – trendline not presented).

Plotting the pruning weights and PCD values of the two Sauvignon Blanc trial sites separately indicated that the relationship may not be the same for the different trellis systems, with a different slope value (Figure 3.2-7). The vigour groups were clustered together, and vines with vigour values between the two groups would be required to improve the strength of this regression.

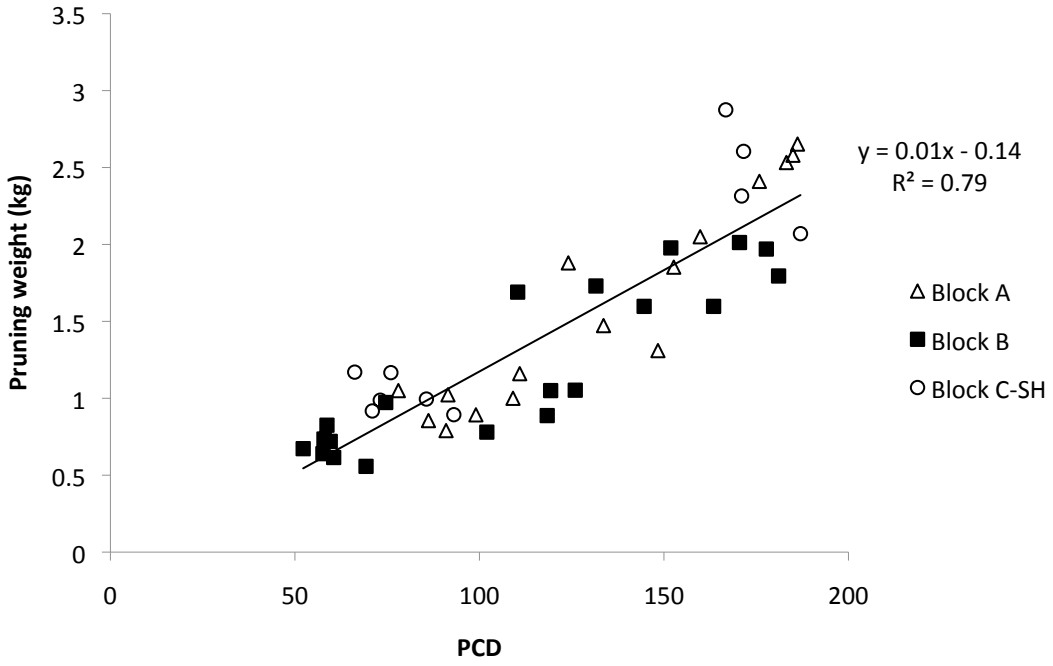

Figure 3.2-6 Pruning weight against PCD for Pinot Noir blocks A, B and Sauvignon Blanc block C-SH in 2005-06. All blocks were trellised to a Scott Henry trellis.

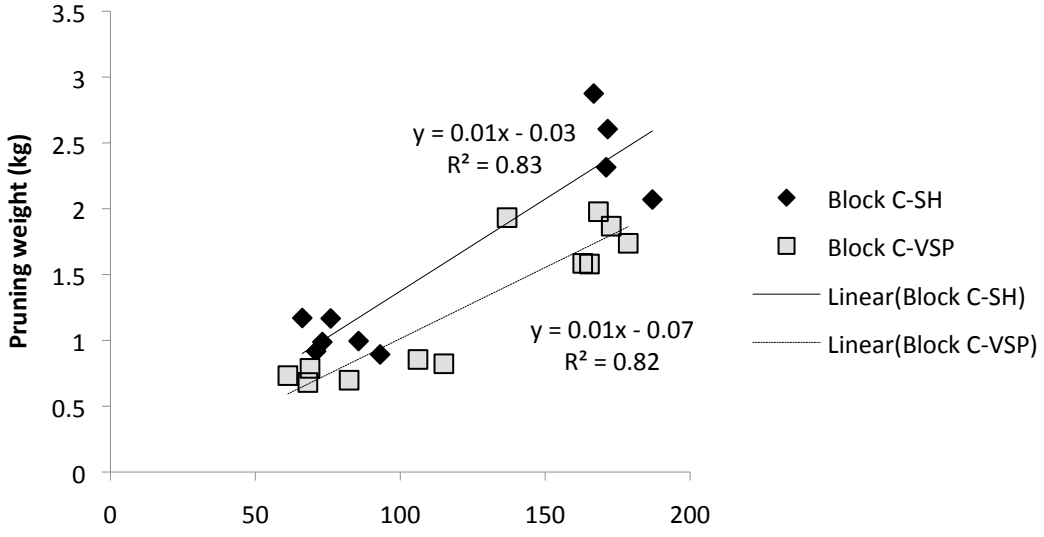

Figure 3.2-7 Pruning weight against PCD for different trellis systems in block C Sauvingon Blanc in 2005-06

Correlations between PCD and other canopy measurements were also examined in block A Pinot Noir, and significant correlations are given in Table 3.2-16 and Table 3.2-17.

*Table 3.2-16 R^2 values for relationships between PCD and vine measured parameters in block A Pinot Noir, 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Pruning weight	Mean cane weight	Upper zone leaf layer number	Fruit zone leaf layer number	Leaf chlorophyll
R^2	0.97	0.87	0.83	0.47	0.53
Sig	***	***	***	**	**

*Table 3.2-17 R^2 values for relationships between PCD and vine measured parameters in block A Pinot Noir, 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Pruning weight	Mean cane weight	Shoot diameter	Trunk diameter	Lateral nodes per shoot	Vigour Scorecard	Sunlight into Wine scorecard
R^2	0.89	0.86	0.81	0.61	0.72	0.82	0.75
Sig	***	***	***	***	***	***	***

3.2d Discussion

Vine vigour and leaf health links

Leaf health can be simply defined as the capacity of a leaf to photosynthesise. By this definition, any factors that impede photosynthesis can be thought of as reducing leaf health. Leaf health in this study was assessed by estimates of chlorophyll concentration, chlorophyll fluorescence measurement and late season leaf retention. Leaf chlorophyll concentration and fluorescence provide direct measures of leaf physiological health, while leaf retention indicates the extent of leaf senescence.

Leaf senescence and chlorophyll concentration were strongly correlated to vine vigour level in all trial sites where they were assessed, and support the idea that stresses that limit shoot growth also impact leaf health. Late season leaf retention was greater in the high vigour zones, both on the older leaves on the main shoot and younger leaves on lateral shoots. While leaf senescence will occur naturally as leaves age (Bertamini and Nedunchezhian, 2002) it is accelerated by the presence of stress, such as water stress and nutrient availability (Lim et al., 2007, Noodén et al., 1997, Yoshida, 2003, Gan and Amasino, 1997). Late season leaf chlorophyll was also increased in higher vigour zones, which is another sign that senescence was less progressed on higher vigour vines (Bertamini and Nedunchezhian, 2002, Bertamini and Nedunchezhian, 2003). Chlorophyll fluorescence measurements indicated that photosynthetic efficiency was also increased with higher vigour, which, along with the increased chlorophyll concentration, may be associated with increased rates of photosynthesis (Candolfi-Vasconcelos et al., 1994). However, when leaf area is non-limiting, increased photosynthesis may not be realised (Poni et al., 1994, Petrie et al., 2000a). The $F_{v/m}$ values indicate that low vigour vines decrease in response to stress, and can be used as a method of monitoring plants for the presence of a stress (Baker, 2008, Maxwell and Johnson, 2000). Therefore, the measurements on leaf retention, leaf chlorophyll and chlorophyll fluorescence indicate that vine leaves will be altered by the stresses faced by that vine, and that these conditions will relate directly to overall vine growth.

There was also evidence of light stress, in the decreased $F_{v/m}$ on leaves in the upper canopy zone, compared to the fruiting zone. Excess exposure to sunlight can cause

damage to the photosynthetic apparatus (Bertamini et al., 2004, Iacono and Sommer, 1996), which may explain this result. While leaf age may cause a similar effect, as observed by Iacono and Sommer (1996) in comparisons apical to midstem and basal leaves, this is probably a minimal factor in this trial since mature leaves were selected for assessment.

Comparing Canopy Assessment Methods

The increases in vine vigour were confirmed by the vine physical measurements. Plant cell density measurement was also effective at determining the relative extent of grapevine canopy growth, and was strongly correlated with vine pruning weight, shoot weight, shoot and trunk diameter, leaf layer number, lateral nodes and the vigour scorecard (Table 3.2-16, Table 3.2-17). Dobrowski et al. (2003) found that aerial imaging using PCD was well correlated to pruning weight in Cabernet Sauvignon on a VSP trellis in California, while Johnson et al. (2003) found similar correlations between satellite-derived normalised differential vegetation index and pruning weight across a number of other varieties, also trained to a VSP trellis. Dobrowksi *et al.* (2003) also found that, with calibration, the relationship between pruning weight and PCD remained consistent across seasons, allowing them to predict pruning weights from veraison imaging results, using the previous season's regression parameters. The lack of calibration, but consistency of response, was evident in the different intercepts but similar slopes of the regression lines between PCD and pruning weight in block A in 2006 and 2007 (Figure 3.2-5). The present study indicates that correlations between PCD and pruning weight on a Scott Henry trellis are also strong. However, the different regression equations between VSP and Scott Henry trellises (Figure 3.2-7) indicate that different trellis systems should be evaluated separately. There appeared to be little difference between cultivars on the same trellis system (Figure 3.2-6), in agreement with Johnson et al. (2003) working on VSP.

Johnson *et al.* (2003) note that once canopy density increases beyond a certain point, vigour indices may become saturated. Nonetheless, in their study a linear correlation was found between leaf area index (LAI) and NDVI with LAI up to 2.8, and cite Montero *et al.* (1999) extending this to an LAI equal to 3.4. While there may have been some degree of interference between the different sections of canopy in this study, leading to the different relationship between the VSP trellis and the Scott Henry trellis in the Sauvignon Blanc trials in 2006 (Figure 3.2-7), the regression was still linear. This suggests that such an effect is minimal, even in the highest vigour vines in this study.

The correlations between plant cell density and point quadrat data where stronger when comparing point quadrat data from the upper part of the canopy, compared to the fruiting zone (Table 3.2-16). Therefore, even though the whole canopy influenced the PCD measurement, the upper canopy zone was still more important than the lower canopy zone when assessing canopy reflectance. In the Pinot Noir in block A, the leaf layers in the fruit zone did not increase between vigour zone 3 and 4, while they did in the upper canopy zone. The point quadrat results from the Sauvignon Blanc showed no significant differences for any measurement of leaf contacts between the vigour zones, however, as in the Pinot Noir, there were significant increases in leaf contacts in the upper canopy zone in high vigour vines. This may be a result of shoot positioning in this vineyard, directing new growth upwards away from the fruit zone.

The resolution of 0.5 m x 0.5 m used in this study where vines are spaced 1.5 m apart, combined with the removal of pixels over interrow spaces, means that each vine is represented by a minimum of 3 pixels. The correlations between PCD and pruning weight were improved when the PCD value of pixels over all four vines in the

measurement panel are used, compared to using the values for each individual vine. Stamatiadis *et al.* (2006), using ground-based sensing equipment, noted a similar improvement in the correlation when a four-vine average was used for the data versus individual vine results.

Harvest yield monitoring can be carried out while running mechanical harvesting units. It is feasible to use the strong vigour index-pruning weight correlation to generate whole farm yield to pruning weight ratios. This proven measure of vine balance may be equally as useful as either of the other measures alone. Alternatively, ground-based yield estimates may be plotted onto the vigour maps to calibrate the yield in different vigour zones.

Bud burst

Percent bud burst increased in higher vigour Pinot Noir vines (Table 3.2-5, Table 3.2-6). Bud burst will be influenced by pruning level and vine capacity (Galet, 2000, Winkler, 1970), hormones mobilised at the end of winter (Lombard et al., 2006) and nutrients (Kliewer et al., 1991), and this was demonstrated by the effect on bud burst from nitrogen fertilisation of vines (Table 4.2-14, Table 4.2-15, Table 4.2-16). With an equal number of buds laid down per cane, as was the case in block B, shoot number will therefore increase with vigour. However, the nodes per cane in block A in 2006 decreased as vigour increased, leading to no significant difference in shoot number per cane. One of the impacts of increased vine vigour is longer internodes (Smart, 1985), and the process of shoot selection and subsequent tying down may lead to less nodes laid down due to less nodes per metre of cane.

Reducing shoot numbers per vine will have the effect of increasing shoot vigour, even on uniform vines, while increasing shoot number will have a devigorating effect (Winkler, 1970). The potential for the increased bud burst to assist in vine devigoration by increasing shoot numbers is lost when less buds are laid down in the pruning of high vigour vines. Where this effect leads to reduced shoots per vine, as in the high vigour VSP trained Sauvignon Blanc vines, this may exacerbate shoot vigour. The Sauvignon Blanc vines demonstrate the benefit of the Scott Henry trellis when training high vigour vines, allowing the vine to produce more shoots than the VSP system.

Pruning weights

Smart and Robinson (1991) suggest that cane weights from 20 to 40 grams indicate moderate vigour, while mean cane weights less than 10 grams represents low vigour and above 60 grams can be classed as having high vigour. The cane weights in all blocks examined in this trial indicate that the vines in the lowest vigour zone can be classed as moderately vigorous. By this measure, shoots from vines in vigour zones 1 and 2 in block A Pinot Noir in both 2005-06 and 2006-07 are in the upper part of the moderate vigour range with mean cane weights between 30 and 47 gm. The higher vigour zones in block A Pinot Noir in both seasons have canes that display excessive vigour, as do zones 4 and 5 in block B Pinot Noir in 2005-06, and the high vigour zones in all trials in block C Sauvignon Blanc in 2005-06 (Figure 3.2-1).

Kliewer and Dokoozlian (2005) offer similar guidelines to those of Smart and Robinson (1991). They do speculate that vines in a cooler climate may require a greater leaf area to fruit weight ratio, although provide no data for this claim. Nonetheless, it is clear that the highest vigour zones would be classed as over-vigorous by current vine balance research.

Periderm development

Periderm browning is linked to wood maturity, and is often delayed on vines that display strong vegetative growth (Winkler, 1970). Water stress may accelerate periderm browning (Matthews et al., 1987). In the current study, vines with high vigour have the highest degree of periderm development (Figure 3.2-4). While the high vigour vines do potentially experience higher levels of midday water stress due to increased leaf area, the hormone response to low water stress is normally associated with reduced growth.

Lignification links to water stress are probably through changes in the levels of plant hormones, and its development coincides with the end of shoot growth (Matthews et al., 1987). Observations by the author indicate that the removal of the shoot tip will accelerate periderm development (unpublished). Higher vigour vines will have a greater percentage of shoots that get trimmed, and also have lateral shoots trimmed. Periderm development that is accelerated by shoot tip removal therefore will have a greater affect on the higher vigour vines. These conclusions are speculative however, and further research is required to better understand periderm browning triggers.

Vigour difference causal factors

The consistency of difference in vigour in block A Pinot Noir and block C, evident in the PCD images and pruning weight data, over the seasons examined in this trial indicates that the changes were soil related. Similar consistency in distribution of vigour has been noted by other researchers using aerial remote sensing to detect vigour differences in grape vines (Hall et al., 2002, Proffitt et al., 2006).

The causes of vigour differences can be wide ranging, however water stress and nutrient stress are the most common causes of reduced vine growth (Keller, 2004). Soil analysis in both block A and block C indicated that nitrogen availability was much greater in the high vigour zones (Refer to the appendix, Table 6.2-1, Table 6.2-4). High vigour vines in both blocks also had a deeper A horizon, with a heavy clay B horizon that may have reduced root penetration (S. Rees, pers. comm.). The increased rooting depth may have increased soil moisture availability. Nitrogen availability can have a large impact on vine growth (Bell and Henschke, 2005, Bell and Robson, 1999, Keller et al., 1998, Keller et al., 2001b, Kliewer et al., 1991, Winkler, 1970), and increased availability can raise chlorophyll concentration (Candolfi-Vasconcelos et al., 1997, Spring and Jelmini, 2002) and photosynthetic efficiency (Cruz et al., 2003, Kitao et al., 2005), as well as photosynthetic rate (Keller, 2004, Keller et al., 1998, Kumar et al., 2002, Vasconcelos et al., 2005). Increased plant nitrogen status will delay leaf senescence, largely directed by increased cytokinin levels (Sakakibara, 2006). A number of other nutritional deficiencies may cause chlorophyll concentration changes. Phosphorus, potassium, magnesium, iron, calcium and manganese deficiencies of grapes may all lead to leaf chlorosis in some form or another (Robinson, 1992, Porro et al., 2001). While there were decreases in soil potassium in block A in low vigour zones, other compounds were at adequate levels. Block C high vigour soils had greater amounts of many nutrients, including potassium, zinc, manganese and iron. Organic carbon also consistently increased with vigour.

3.2e Conclusions

The PCD values derived from remote sensing using aerial infrared imaging were well correlated to many ground based assessments of vigour, particularly pruning weight, as has been found by other researchers (Carnevali et al., 2009, Johnson et al., 1996, Dobrowski et al., 2002, Dobrowski et al., 2003, Hall et al., 2008, Johnson et al., 2003). Therefore, it may be possible to use any one of these measures to help identify different

vigour zones. This includes the use of scorecard systems, which provide rapid analysis of vigour.

Late season leaf chlorophyll concentration and leaf retention are also correlated to PCD, and may also be useful as indicators of vigour.

Vine canopy density and shoot vigour will alter the grape microclimate and influence grape and wine attributes (Cortell et al., 2005, Cortell et al., 2007a, Cortell et al., 2007b, Smart, 1985). Altered photosynthetic capacity of the canopy as a result of changing leaf area and leaf health may also interact with fruit yield to change the ripening rate of the vine. The confirmation that shoot vigour differences were significant between the vigour zones selected in this trial indicate that grape and wine attributes may also be altered in the different vigour zones. This will be investigated in the following chapters.

3.3 The impact of variable vine vigour on fruit composition and yield in grapevine (*Vitis vinifera* L.) cvs Pinot Noir and Sauvignon blanc

3.3a Introduction

The extent of vegetative growth of a grapevine can be an important factor in determining grape yield and fruit attributes, and in turn the attributes of the wine produced from those grapes.

Yield can be affected by vigour level. Increased vine vigour can alter the microclimate in the fruit zone, decreasing light penetration (Smart, 1985) and air movement (Thomas et al., 1988). Dry (2000) reviewed the influence of canopy growth on yield and fruitfulness, and identified the primary importance of temperature and light on the development of buds, with increases in both light exposure and temperature of the buds being positively correlated to fruitfulness. Additionally, low light and reduced airflow may lead to reduced fruit set (Dry and Loveys, 1998, Smart and Robinson, 1991). Nutritional deficiencies and vine stress, which will reduce vine vegetative growth, may also lead to poor fruit set. Studies linking yield to vigour have found that in some circumstances, yield increases with vigour (Proffitt et al., 2006, Clingeleffer and Sommer, 1995), which has led to suggestions that measures of vine vigour can be used as proxy measures for yield (Hall et al., 2002). However other researchers have reported that increasing vigour may decrease yields, or have no correlation (Cortell et al., 2007b).

Berry exposure can increase total soluble solids (TSS), although if excessive it may delay sugar accumulation (Bergqvist et al., 2001). Cortell et al (2008) report that increasing vigour did not consistently alter TSS levels. More consistently observed is an increase in titratable acids with higher vigour (Cortell et al., 2008), Malic acid degradation will be increased by greater fruit temperature (Keller, 2010), which will lower titratable acidity measurements. Potassium movement into the fruit can also increase in higher vigour vines as a result of increased shading (Smart, 1985, Keller, 2010), which will raise pH at a given titratable acidity (Jackson, 1994, Keller, 2010).

Flavan-3-ol compounds and proanthocyanidin polymers (tannins) (Adams, 2006), are both increased by higher fruit exposure (Ristic et al., 2007, Bergqvist et al., 2001, Keller and Hradzina, 1998, Spayd et al., 2002, Cortell and Kennedy, 2006). Studies examining the impact of vine canopy growth on phenolics have found that increasing vigour produces grapes with lower tannin concentration (Cortell et al., 2005, Cortell et al., 2008, Smart, 1985, Lamb et al., 2004).

Anthocyanin concentration of red grapes is less affected by shading than phenolics (Cortell and Kennedy, 2006, Ristic et al., 2007), however the composition of the constituent anthocyanins is likely to change (Cortell and Kennedy, 2006, Keller and Hradzina, 1998). Temperature changes due to sunlight are more important than the light itself for anthocyanin production, (Spayd et al., 2002, Bergqvist et al., 2001, Deis et al., 2009). Cortell et al (2007b) reported that increasing vine vigour in Pinot Noir was linked to a trend towards decreased anthocyanin in one trial, but there was no change in another. Trials where high vigour vine growth was reduced through vineyard floor management have led to increased anthocyanin concentration (Wheeler et al., 2005).

One nutrient of particular importance in determining vine vigour is nitrogen (Keller, 2004), and increases in nitrogen can have direct impacts on fruit chemistry, in addition to

the changes resulting from increasing canopy growth. Higher levels of nitrogen can reduce phenolics and anthocyanins (Keller and Hradzina, 1998, Keller et al., 1999, Pirie and Mullins, 1976). Fermentation dynamics can be influenced by nitrogen quantities available to the yeast. Yeast assimilable nitrogen (YAN) levels may reflect the nitrogen status of the vine as a whole. YAN levels found in musts cover a broad range. Nitrogen availability may drive vine growth, and as a result high vigour vines will often have a elevated tissue nitrogen concentration (Hall et al., 2002). The link between vigour and berry nitrogen is so strong that at one stage, it was proposed that vine nitrogen status could determined by measuring must arginine levels (Bath et al., 1991, Kliewer and Cook, 1974), although it was never adopted as a standard. Arginine is also one of the major amino acids contributing to yeast assimilable nitrogen (Bell and Henschke, 2005, Henschke and Jiranek, 1993). Therefore, increasing yeast assimilable nitrogen with increasing vigour is expected, and has been observed (Cortell et al., 2008).

Increased vigour can increase the occurrence of infection by fungal pathogens such as botrytis, due to the higher leaf area in the fruit zone decreasing air movement through the fruit zone, which increases drying time of dew or precipitation, and decreases the coverage of preventative sprays (Thomas et al., 1988, English et al., 1989, Smart, 1991). Further increasing this susceptibility is reductions in stilbene concentration, a group of phenolic compounds which have antifungal properties (Adrian et al., 2000, Keller, 2010), and are also produced in response to UV light.

It is very common for vineyard management units to incorporate areas with different vigour levels (Bramley and Lamb, 2003, Hall et al., 2002). Understanding the impact of vigour on fruit and yield will allow managers to determine the optimal management programs for meeting wine quality objectives.

The objective of this trial was to investigate the effect of vine vigour level on the yield components and fruit attributes of Pinot Noir and Sauvignon Blanc grapes. Canopies had previously been assessed to determine the range of vigour levels present, and link this to vine leaf health. This data is presented in the preceding chapter, and confirmed that the vigour levels in the trial were significantly different, and were associated with more leaves, denser canopies and greater vegetative growth (section 3.2, p. 40). Identifying the impact of vigour differences on yield and fruit chemical attributes will aid in determining the impact of harvesting heterogonous vineyards together, and in identifying the optimal viticultural practices for producing quality wines. The impact of vigour on Botrytis infection was also assessed, since this can have large implications on wine quality.

3.3b Materials and Methods

Trial vineyard description

Four trial sites were used in the 2005-06 season. Two were Pinot Noir blocks (block A and block B), and two were in Sauvignon Blanc (block C VSP (vertical shoot positioned trellis) and block C SH (Scott Henry trellis)).

In 2006-07, more measures were made in block A Pinot Noir and also in block C Sauvignon Blanc on a Scott Henry trellis.

For further details about site layout, please refer to the general materials and methods section (p. 17).

*Table 3.3-1 Fruit and yield analyses performed in vigour trial blocks. Abbreviations - TA - Titratable Acids; TSS - Total Soluble Solids; YAN - Yeast Assimilable Nitrogen. * - more information on this analysis below the table*

Trial	*Analysis performed*	*Date*	*Reference page*
Block A Pinot Noir	Veraison progression*	26/02/07	
	Yield and yield components*	13/04/06 18/04/07 9/04/08	34
	Juice pH, TA and TSS	16/04/06 19/04/07 9/04/08	33
	YAN	12/05/06 25/05/07 11/04/08	34
	Malate	12/05/06	33
	Berry Extract Analysis*	19/06/08	34
	Botrytis incidence*	13/04/06 18/04/07 9/04/08	
Block B Pinot Noir	Yield and yield components*	7/04/06	34
	Botrytis incidence*	7/04/06	
Block C VSP Sauvignon Blanc	Yield and yield components*	26/03/06	34
	Juice pH, TA and TSS	28/03/06	33
Block C SH Sauvignon Blanc	Yield and yield components*	26/03/06	34
	Juice pH, TA and TSS	28/03/06	33
Block C-07 Sauvignon Blanc	Yield*	27/03/07	34
	Juice pH, TA and TSS	28/03/07	33
	YAN	28/03/07	34

Vine Canopy Measures

Regression analyses were conducted between yield per vine in all blocks in 2005-06, and plant cell density (PCD). The PCD values were derived from aerial infrared imaging, as described in the general materials and methods (p. 25).

Yield:Pruning weight ratios were calculated using block pruning weights in 2005-06 and 2006-07 in block A Pinot Noir, and in 2005-06 in block B Pinot Noir and both block C VSP and block C SH Sauvignon Bland trials. Pruning weights were collected using the protocols outlined in the materials and methods (p. 28). Shoot counts used in yield component analysis were also taken from pruning weight data.

Leaf nitrogen concentration was assessed in the Sauvignon Blanc block in 2007 following the method outlined in the general materials and methods (p. 29).

Veraison progression

Veraison progression was assessed in block A in 2006-07 in vigour zones 1, 2 and 4. Zone 3 was omitted due to a lack of time available. Ten shoots were selected at random in each measurement panel, and the percentage of berries per bunch that displayed anthocyanin over 50% of the berry surface was estimated. Berry counts were also carried out, to ensure the visual assessor was "calibrated".

Yield and yield components

Within each trial and each season, fruit was harvested from all vigour zones on the same day. Harvest date was selected on the basis of berry sampling, aiming for a total soluble solids (TSS) level of around 24° Brix for both Sauvignon Blanc and Pinot Noir in all seasons. All fruit was removed from the four vines of each measurement panel and weighted, and the number of bunches counted.

Average bunch weights were assessed in all seasons. Berry weights were assessed in block A Pinot Noir in 2006 and 2008. Berry samples were taken by random sampling of a couple of berries from every bunch, once picked. Counting of bunches was carried out at the same time. Berries in the sample were counted and weighed to get an average berry weight.

Botrytis infection

Bunches with visible botrytis infection were removed from the main sample at harvest. Botrytis infected bunches were bulked together and weighed in 2005-06 in block A and B Pinot Noir trials. In block A Pinot Noir during the 2006-07 and 2007-08 harvests bunches were counted instead of being weighed since it was observed that severe botrytis infection significantly reduced bunch weight.

3.3c Results

Yield and yield components

There were no significant differences in the yield per vine or in the bunches per vine between vigour zones in any year of the trial in block A Pinot Noir, although there was a significant difference between the seasons, with 2008 having a greater yield than 2006 and 2007 (Table 3.3-2).

Yield in block B Pinot Noir showed a significant increase as vigour level rose (Table 3.3-3).

The plant cell density (PCD) values of the measurement panels in blocks A and B Pinot Noir in 2005-06 were within a similar range as can be seen in Figure 3.3-1, indicating that the vegetative growth was comparable in both areas (Dobrowski et al., 2002). There was a positive correlation between yield and PCD in block B, however there was none in block A (Figure 3.3-1).

Bunches per shoot, bunch weight, and shoots per vine all increased in response to vigour increases in block B.

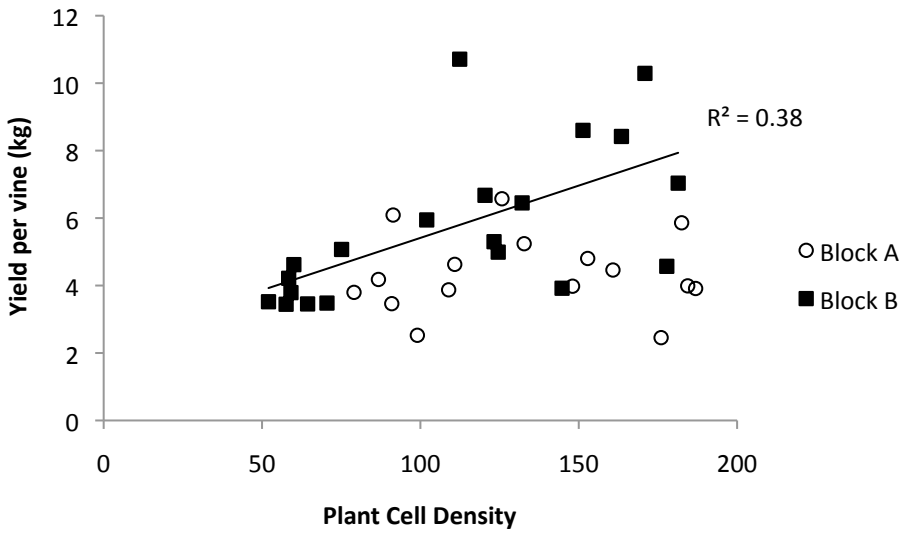

Figure 3.3-1 Panel plant cell density (PCD) against yield per vine in Pinot Noir blocks A and B in the 2005-06 season. Trendline is for block B, where there was a significant correlation between PCD and yield (P=0.003). There was no significant correlation between PCD and yield in block A Pinot Noir.

Bud burst percentage was increased in the higher vigour zones of both block A Pinot Noir and B Pinot Noir (Table 3.2-5, Table 3.2-6) but this did not produce a greater shoot count per vine (Table 3.3-3, Table 3.3-2).

Bunch count per vine was increased by higher vigour in block B, primarily as an increase in bunches per shoot Table 3.3-3. Block A showed no change in bunches per vine in response to vigour in either 2005-06 or 2006-07, although bunches per shoot was marginally decreased in 2005-06 in the higher vigour zones.

Bunch weight did not vary significantly in response to vigour in block B Pinot Noir, although there was an increasing trend, and bunch weight did not change significantly in block A Pinot Noir in any year of the trial.

*Table 3.3-2 Block A yield components in all years of the trial 2006. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference). (No berry weight data for 2006-07; no shoot count data for 2007-08)*

Season	Vigour	Yield per vine (kg)	Bunches per vine	Bunches per shoot	Bunch wt (g)	Berry wt (g)	Shoot count
2005-06	1	3.49	34.4	2.0a	103	1.19	17.2
	2	4.64	36.1	1.9ab	129	1.18	19.3
	3	5.27	38.2	1.7ab	137	1.21	22.6
	4	4.05	33.4	1.6b	120	1.13	20.7
	Sig	n.s.	n.s.	*	n.s.	n.s.	n.s.
2006-07	1	3.75	28.8	1.5	131	-	19.1
	2	4.83	32.1	1.5	154	-	20.9
	3	3.98	28.0	1.4	146	-	20.4
	4	3.55	28.8	1.4	125	-	19.9
	Sig	n.s.	n.s.	n.s.	n.s.	-	n.s.
2007-08	1	5.80	37.2	-	157	1.3	-
	2	6.25	38.2	-	164	1.4	-
	3	5.86	38.9	-	151	1.3	-
	4	4.73	33.6	-	140	1.4	-
	Sig	n.s.	n.s.	-	n.s.	n.s.	-

*Table 3.3-3 Yield components for block B in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Yield per vine (kg)	Bunches per vine	Bunches per shoot	Shoots per vine	Bunch wt (g)
1	3.65a	38.9a	1.61a	24.1	94
2	4.25a	37.1a	1.52a	24.8	115
3	5.73ab	46ab	1.59ab	29.0	124
4	7.42b	51.8b	1.85bc	27.6	141
5	7.58b	53.4b	2.2c	24.6	140
Sig	*	*	**	n.s.	n.s.

Yield in Sauvignon Blanc vigour trials (block C VSP, block C SH and block C 07) increased in response to greater vine vigour (Table 3.3-4, Table 3.3-5, Table 3.3-6). In 2005-06 the number of bunches per vine was higher on high vigour vines, due to increasing bunches per shoot as well as more shoots per vine. The average bunch weight and berry weight also increased).

There was a clear difference in yield due to trellis type (Figure 3.3-2). Yields from vines trellised to a Scott Henry system were higher than yields from the VSP vines, although the PCD values indicate that the amount of canopy was comparable. A heteroscedastic t-test indicated that this difference was significant (P=0.003).

There was an increase in bunches per vine and bunches per shoot in both Scott Henry and VSP trellised vines. Increasing vigour in Sauvignon Blanc vines trellised to a Scott Henry trellis system led to an increase in shoot number (Table 3.3-4). Therefore, while bunches per shoot did not change between trellis systems, bunches per vine increased on vines trained to the Scott Henry trellis. While shoots per vine increased in the Scott Henry trellised vines, shoots per vine decreased from low vigour to high vigour in the VSP vines (Table 3.3-4, Table 3.3-5).

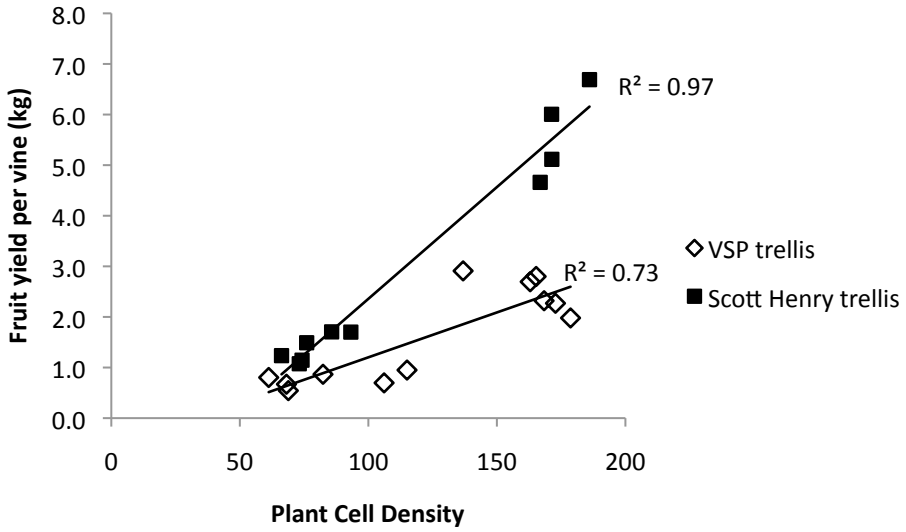

Figure 3.3-2 Plant cell density plotted against yield per vine in 2005-06 in two Sauvignon Blanc vigour trials comparing yields of vines on a VSP trellis with a Scott Henry

*Table 3.3-4 Yield components in Scott Henry trellised Sauvignon Blanc vigour trial in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Yield per vine (kg)*	*Bunches per vine*	*Bunches per shoot*	*Shoots per vine*	*Bunch wt (g)*	*Berry wt (g)*
Low	1.39a	26a	0.88a	25.3a	54a	1.41a
High	5.62b	49b	1.51b	31.3b	116b	1.55b
Sig	***	***	***	**	***	*

*Table 3.3-5 Yield components in Sauvignon Blanc VSP trellised vigour trial in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Yield per vine (kg)*	*Bunches per vine*	*Bunches per shoot*	*Shoots per vine*	*Bunch wt (g)*	*Berry wt (g)*
Low	0.76a	18.9a	0.86a	21.5b	40a	1.24a
High	2.50b	23.3b	1.37b	16.6a	107b	1.35b
Sig	***	*	***	**	***	*

*Table 3.3-6 Yield per vine in block C 07 Sauvignon Blanc with changing vigour level. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Yield per vine (kg)*
Low	1.3
High	2.5
Sig	**

Yield to Pruning Weight Ratios

Block A showed a very significant decrease in yield to pruning weight ratios as vine vigour increased (Table 3.3-7). In contrast, block B showed no significant difference in yield to pruning weight ratio (Table 3.3-8). The Sauvignon Blanc vines also increased yield to pruning weight ratio as vigour increased (Table 3.3-9).

*Table 3.3-7 Yield to pruning weight ratios in block A Pinot Noir for 2006 and 2007. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*2006 Yield to pruning weight ratio*	*2007 Yield to pruning weight ratio*
1	3.9bc	5.0b
2	4.2c	4.5b
3	3.0b	2.4a
4	1.6a	1.4a
Sig	**	***

*Table 3.3-8 Yield to pruning weight ratio, block B Pinot Noir in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Yield to Pruning weight ratio*
1	5.7
2	5.4
3	6.2
4	4.2
5	4.2
Sig	n.s.

*Table 3.3-9 Yield to pruning weight ratios, Sauvignon Blanc vigour trials in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*VSP*	*Scott Henry*
Low	1.00a	1.42a
High	1.42b	2.13b
Sig	*	**

Veraison progression

Veraison progression in block A in 2007 showed no significant differences between vigour zones (Table 3.3-10).

*Table 3.3-10 Relative veraison development between vigour zones in block A, 2006-07. Note that zone 3 was not assessed for veraison progression. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Basal bunch Berries over 50% coloured (%)*	*Distal bunch berries over 50% coloured (%)*	*% of total bunches with berries over 50% coloured*
1	76	62	71
2	80	68	76
4	82	65	75
Sig	n.s.	n.s.	n.s.

Fruit Chemical Attributes

Block A Pinot Noir did not exhibit any significant difference in total soluble solids (TSS) between vigour zones in either 2006 or 2007, however in 2008 vigour zones 2 and 3 had significantly lower TSS (Table 3.3-11). The highest vigour zone had higher TSS than zones 2 and 3, although not significantly higher than the lowest vigour zone. TSS levels in the 2007-08 season were also the most strongly correlated to yield, with a less robust

correlation in 2005-06 and no correlation at all in 2006-07 (Table 3.3-12).

Significant pH changes associated with vigour zone in block A were seen only in 2006, with an increase in pH in the highest vigour zone (Table 3.3-13). There was a trend towards a similar relationship in the 2008 season (P=0.054). When repeated measures analysis of the results for all three years was carried out, the pH of the highest vigour zone was significantly higher than other vigour levels.

In block A, titratable acids (TA) rose with increasing vigour in all seasons (Table 3.3-13). TA was highest in the high vigour zone in all years of the trial, even though the pH was also higher at this vigour level. This was seen individually in all seasons, and, as expected, also in repeated measures analysis.

*Table 3.3-11 Total soluble solids (°Brix), block A, in all years. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*2005-06 Brix*	*2006-07 Brix*	*2007-08 Brix*
1	24.5	26.6	23.3ab
2	24.2	26.2	22.8a
3	24.3	26.0	22.6a
4	25.1	25.1	23.9b
Sig	n.s.	n.s.	*

*Table 3.3-12 Regression between yield and juice Brix levels in block A Pinot Noir across three seasons. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	2005-06	*2006-07*	*2007-08*
R^2	0.31	0.06	0.51
Sig	*	n.s.	**

*Table 3.3-13 Wine pH and titratable acidity (in g/L) in block A Pinot Noir, in all years and with all data analysed by a repeated measures analysis. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Season	*2005-06*		*2006-07*		*2007-08*		*All seasons*	
Vigour	pH	TA	pH	TA	pH	TA	pH	TA
1	3.4a	6.6a	3.6	5.4a	3.4	6.2a	3.5a	6.1a
2	3.4a	6.9ab	3.5	5.5a	3.4	6.7a	3.4a	6.4ab
3	3.4a	7.3bc	3.6	6.0b	3.4	6.8ab	3.5a	6.7b
4	3.5b	7.6c	3.6	6.9c	3.5	7.5b	3.5b	7.3c
Sig	**	**	n.s.	***	n.s.	*	**	***

Enzymatic analysis of juice from block A Pinot Noir in 2006-07 indicated that malate levels rose with vigour zone in a similar manner to the titratable acids (Table 3.3-14).

*Table 3.3-14 Malate levels in block A Pinot Noir, 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Malate (g/L)*
1	2.7a
2	2.7a
3	3.1b
4	3.7c
Sig	***

In the two Sauvignon Blanc trials in 2005-06, TSS significantly decreased with increasing vigour, while pH and titratable acidity both increased (Table 3.3-15, Table 3.3-16). The

juice from block C-07 Sauvignon Blanc vines in 2006-07 showed the same vigour effects (Table 3.3-17). Malate levels in that season showed no differences between treatments.

*Table 3.3-15 Block C VSP trellised Sauvignon Blanc vigour trial juice chemistry in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*TSS (Brix)*	*pH*	*Titratable Acids (g/L)*
Low	22.1b	3.00a	8.8a
High	20.9a	3.07b	10.0b
Sig	**	***	***

*Table 3.3-16 Block C Scott Henry trellised Sauvignon Blanc vigour trial juice chemistry in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*TSS (Brix)*	*pH*	*Titratable Acids (g/L)*
Low	21.5b	2.98	10.4
High	19.2a	3.04	12.5
Sig	***	***	***

*Table 3.3-17 Block C-07 Sauvignon Blanc vigour trial juice soluble solids, pH, titratable acidity and malate level in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*TSS (Brix)*	*pH*	*Titratable Acids (g/L)*	*Malate (g/L)*
Low	22.7	3.28	7.7	2.1
High	21.1	3.38	6.8	2.0
Sig	***	**	*	n.s.

Yeast assimilable nitrogen

Yeast assimilable nitrogen (YAN) rose with vigour in block A in all years, although there were no significant differences between the two lowest vigour zones (Figure 3.3-3). YAN also rose in the higher vigour zone in the Sauvignon Blanc trial, being 102 mg N/L in juice of low vigour vine fruit compared to 133 mg N/L in the juice of fruit from high vigour vines. The YAN concentration was correlated to the leaf nitrogen concentration (Figure 3.3-4).

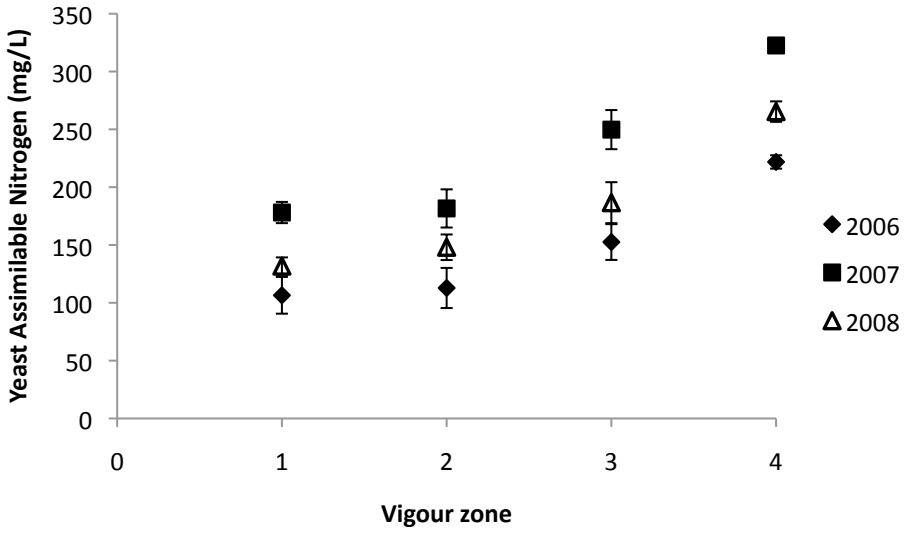

Figure 3.3-3 Yeast assimilable nitrogen in block A over three seasons. Error bars represent standard errors of the mean.

*Table 3.3-18 Yeast assimilable nitrogen in Sauvignon Blanc juice from different vigour zones in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*YAN (mg N/L)*
Low	102a
High	133b
Sig	**

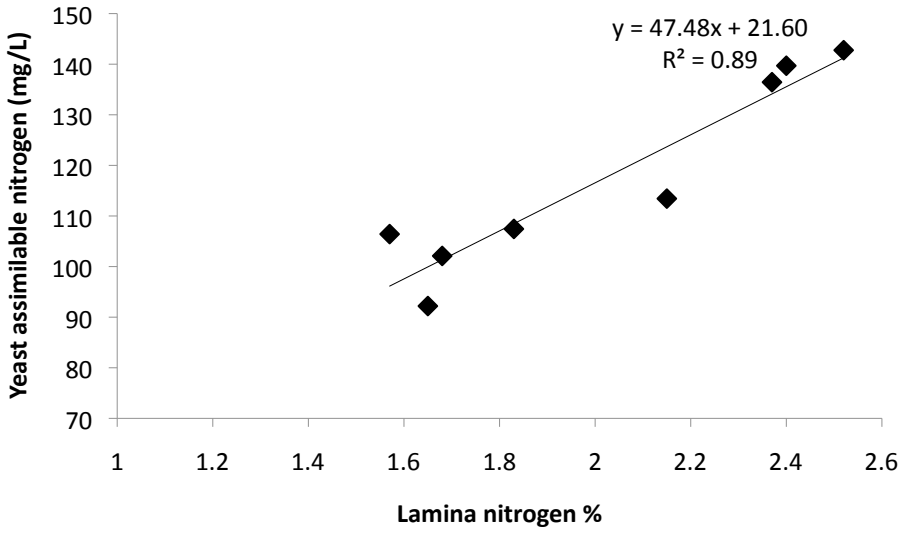

Figure 3.3-4 Leaf lamina nitrogen concentration and YAN concentration from Block C-07 Sauvignon Blanc in 2006-07.

Berry Phenolic and Anthocyanin content

Berry homogenate tannin content was significantly less in berries from vigour zone 4 in block A Pinot Noir (Table 3.3-19), but not significantly different at any other vigour level, when assessed both on a per berry basis and on a per weight of homogenate basis.

Anthocyanin levels showed no significant variation with vigour.

*Table 3.3-19 Berry homogenate extract anthocyanin and tannin content, block A Pinot Noir, 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Tannin mg per berry	Tannin mg/g homogenate	Anthocyanin mg per berry	Anthocyanin mg/g homogenate
1	9.9b	7.3b	0.80	0.60
2	10.4b	7.2b	0.82	0.57
3	9.7b	7.3b	0.77	0.58
4	8.9a	6.5a	0.82	0.61
Sig	**	**	n.s.	n.s.

Botrytis

There was a trend towards higher botrytis infection in vigour level 3 in block A Pinot Noir in all seasons, although this was only significant in 2007 (Table 3.3-20).

*Table 3.3-20 Botrytis cinerea infection levels in block A Pinot Noir, all years. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	2006 Rejected bunches total weight per vine (g)	2007 Infected bunches per vine	2008 Infected bunches per vine
1	0.40	2.2a	3.8
2	1.67	4.4ab	8.6
3	2.69	8.1b	11.3
4	2.20	3.5ab	3.4
Sig	n.s.	*	n.s.

Correlations between fruit physical measures and vine PCD indicated that there were few links between canopy characteristics and disease levels in block A Pinot Noir, with no significant regression with PCD in either 2006 or 2007 (Table 3.3-21). PCD values were not available for 2008, however the correlations with botrytis for this year indicated that yield per vine and bunches per vine had a greater impact on disease levels than vine vigour.

In block B PCD had a significant relationship to the total weight of bunches harvested that were affected by botrytis (Table 3.3-22). Aside from bunch counts, no other measurements had a significant relationship with botrytis disease levels.

*Table 3.3-21 Botrytis bunch counts and R^2 values for regressions between botrytis damage and PCD, yield per vine, bunch weight and bunches per vine in 2005-06, in block A Pinot Noir. In 2005-06 botrytis damage was assessed as total weight of infected bunches, while in 2006-07 and 2007-08 it was assessed as the number of infected bunches. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Season	PCD	Yield per vine	Bunch weight	Bunch count
2005-06	0.04 (n.s.)	0.72 (***)	0.57 (***)	0.30 (*)
2006-07	0.00 (n.s.)	0.08 (n.s.)	0.02 (n.s.)	0.10 (n.s.)
2007-08	-	0.45 (**)	0.00 (n.s.)	0.33 (*)

*Table 3.3-22 Weight of Botrytis infected bunches and R^2 values for regressions with PCD and yield per vine, bunch weight and bunches per vine in 2005-06, in block B Pinot Noir. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	PCD	Yield per vine	Bunch weight	Bunch count
Botrytis weight	0.51 (***)	0.07 (n.s.)	0.00 (n.s.)	0.17 (*)

3.3d Discussion

Vigour and yield relationship

Vigour and yield relationships in the blocks examined in this study were not consistent between trials. Block A Pinot Noir showed no linear relationship between vigour and yield in any season however there was a positive correlation between vigour and yield in block B (Figure 3.3-1). A positive correlation was also observed between yield and vigour in the Sauvignon Blanc trial sites in all seasons (Figure 3.3-2). The different reactions observed here are also observed in other studies. Cortell (2005), working with Pinot Noir, found that moderate vigour levels gave rise to higher yields than both lower and higher vigour vines, however others have seen that yield increases with vigour (Clingeleffer and Sommer, 1995).

This disparity in results reflects the complex relationships between canopy growth and yield. Increasing vigour from a low level to a moderate level can lead to increases in carbohydrates and nutrients, which may increase yields (Clingeleffer and Sommer, 1995), however decreases in light exposure of buds and air movement through the canopy as vigour gets higher still can decrease fruitfulness and fruit set (Dry, 2000, Dry and Loveys, 1998, Smart and Robinson, 1991).

The components of yield that contributed to the differences in vigour observed in this study were different between the Pinot Noir and the Sauvignon Blanc. While the Pinot Noir vines in block B had more bunches per shoot in higher vigour areas, the Sauvignon Blanc vines had more shoots per vine, more bunches per shoot, more berries per bunch and greater berry weights in higher vigour areas.

Yield per vine is a function of the shoots per vine, bunches per shoot and bunch weight (Dry, 2000). Given the number of steps involved in determining these factors, a simple linear relationship between yield and vigour may not be expected. Increased number of shoots per vine may increase the total bunches per vine. Higher vigour vines in block A Pinot Noir and block B Pinot Noir had an increased percentage bud burst (Table 3.2-5, Table 3.2-6), however shoots per vine did not vary. Internode length increases with vigour (Smart, 1985) and when cane pruned vines have canes tied down to fill the fruiting wire node number per vine may decrease on higher vigour vines, meaning the length of cane is constant between vigour zones. This may offset the increase in budburst.

Excessive shading can reduce bunches per shoot and flowers per bunch (Dry, 2000). It is likely that the high density of the canopies in the higher vigour zones in the block A Pinot Noir trial was leading to excessive shading of buds, which in turn was reducing bunches per shoot (Table 3.3-2). Conversely, bunches per shoot were increased in the Pinot Noir in block B, even though the vigour level, as assessed by PCD, was similar in both zones (Table 3.3-3). One difference between these two trial sites was aspect. Block A was on a moderate slope to the south, while block B was more level. This difference may have altered the level of exposure enough to create the changing relationships. The results from this study indicate the difficulties in using aerial remote sensing to forecast

yields, which has been proposed as one use of this type of monitoring (Hall et al., 2002), without good ground measurements to calibrate the image.

Yield to pruning weight ratio

Relationships between yield to pruning weight (Y:PW) ratios and vigour differed between the different trial sites. In the Pinot Noir trial blocks the Y:PW ratio decreased as vigour increased in block A but did not differ significantly between vigour zones in block B. The Y:PW of both Sauvignon Blanc trials in 2005-06 increased with vigour. Y:PW values around 5-10 are said to indicate moderate vigour and optimal balance, while vines with excessively low vigour will have values above this range, and overly vigorous vines below this range (Smart and Robinson, 1991, Kliewer and Dokoozlian, 2005). The optimal range is linked to leaf area to fruit weight ratios of 0.8 – 1.2 m^2 leaf area per kg of fruit. In warm climates this is generally sufficient to get fruit to ripen, although more may be required in cooler climates such as Tasmania (Kliewer and Dokoozlian, 2005).

Using these values as a guide, all of the trial sites examined may be classified as having sufficient to excessive canopies for the fruit loads present. The imbalance between canopy and fruit in the high vigour vines in block A Pinot Noir was more pronounced, since the yields were not increasing with vigour as they were in block B Pinot Noir. The block C Sauvignon Blanc vines became more balanced as vigour increased, and the Sauvignon Blanc vines on the Scott Henry trellis were closer to the optimum than vines on the VSP trellis.

Fruit maturation and chemistry

Soluble solids were not affected by vigour level in any year of the trial in block A Pinot Noir except 2008, when the high and low vigour vines had higher sugars at harvest than the intervening levels of vigour. In 2007, veraison progression did not differ between vigour levels in block A either. Sugar accumulation may be reduced if leaf area is not adequate for ripening (Petrie et al., 2000b, Jackson, 1986, Kliewer and Dokoozlian, 2005). There was no evidence that low vigour vines had leaf areas below the adequate level in this trial, consistent with the range of Y:PW observed (Kliewer and Dokoozlian, 2005, Smart and Robinson, 1991).

The high vigour Sauvignon Blanc vines had a lower sugar level at harvest in both the 2005-06 and 2006-07 seasons. As with the Pinot Noir, the Y:PW ratio indicated that there was more than sufficient canopy for the crop load present. However, berry weights of fruit from higher vigour zones also increased, and although sugar concentration may have been less in the fruit of high vigour vines sugar content per berry may not have changed with vigour. The simple conversion used here, where 1°Brix was equivalent to 1% sugar, indicated that there was little difference in the quantity of sugar per berry (Table 3.3-15, Table 3.3-16).

Vigour impacts on sugar level have been observed by other authors, but with varying relationships. Cortell (2008) found that in some cases sugars increased with vigour and in others they decreased. Water stress will reduce vine growth, and may also lead to earlier ripening of fruit as a result of the production of abscissic acid (Hartung et al., 2005, Robinson and Davies, 2000). The soil analysis indicated that there is a greater soil volume in the high vigour areas of block A Pinot Noir and Sauvignon Blanc blocks C VSP and C 07, which is likely to provide increased access to water (S. Rees, pers. Comm.; see Table 6.2-1 and Table 6.2-4 in Appendix for A horizon depth).

Juice pH increased in the highest vigour zone in block A, with no significant differences in the three lower vigour levels. Titratable acidity also increased as vigour increased, with

a rise from the lowest to the highest vigour level. Malate concentration in the Pinot Noir in 2007 rose significantly as vigour increased, which will contribute to the increase in titratable acids (Jackson, 1994). Titratable acid levels and pH also increased with vigour in the block C VSP and SH Sauvignon Blanc trials in 2005-06. Titratable acidity has been shown to decrease in response to exposure of berries to sunlight and to increased berry temperature (Bergqvist et al., 2001, Spayd et al., 2002), which will stimulate increased degradation of malic acid (Keller, 2010). Trials with nitrogen fertilisation, which can increase vine vigour, often find an increase in TA as a result of fertilisation (Choné et al., 2006, Bell and Henschke, 2005). Malic acid is a weaker acid than tartaric, so juices with a higher proportion of malic acid will tend to have a higher pH. Potassium cations can balance the malate anions, and if potassium levels are high in the juice pH will be high. Potassium levels have been shown to be positively correlated to malate levels (Bravdo and Hepner, 1986), and to be increased by shading (Morrison and Noble, 1990) therefore it may be that an increase in potassium contributed to the higher pH, although this was not measured in this study.

In 2006-07 in block C Sauvignon Blanc TA increased with low vigour, and there was no difference between malate levels. Vine water stress can lead to a reduced moisture content in the fruit. Tartaric acid is formed prior to veraison, and remains stable from then until harvest when measured per berry will only decrease slightly per berry (Keller, 2010). As a result, dehydration can increase the concentration of acids (Fang et al., 2011). Under these conditions, juice TA would increase.

Yeast assimilable nitrogen was also seen to rise strongly with vigour level, in all trials where it was measured. The Pinot Noir in block A was strongly influenced by season, however the relative impact of vigour zone remained consistent (Figure 3.3-3). The higher YAN was closely linked to the nitrogen status of the vines in the Sauvignon Blanc in block C in 2007 (Figure 3.3-4), and also increased with yield. Other trials associated with this thesis indicated that the same could be expected in Pinot Noir. Other researchers have also identified a strong link between amino acid concentration, particularly arginine, and overall vine nitrogen status (Bath et al., 1991, Juhasz et al., 1984, Kliewer, 1991, Kliewer and Cook, 1974).

Adequate YAN levels for complete fermentation vary between red and white ferments (Bell and Henschke, 2005), and between yeast strains (Jiranek et al., 1995a, Gardner et al., 2002). Although research reports a range of minimum YAN concentrations from 70 to 267 mg/L for successful fermentation (Bell and Henschke, 2005), the most common management recommendation for minimum YAN levels to ensure complete fermentation is 140 mg N/L. There is likely to be a significant portion of YAN unmeasured in the red ferments, since grape solids hold nitrogen that is made available to yeast during fermentation (Bell and Henschke, 2005, Yokotsuka and Singleton, 1996). Therefore the low vigour vines in the Pinot Noir with low YAN levels may require nitrogen supplementation under some circumstances, and the juices of all the Sauvignon Blanc trials indicates that there is a high likelihood that supplementation of the juice in the winery may be required for a complete fermentation. The Sauvignon Blanc vine leaf analysis indicated that these vines were not suffering from a nitrogen stress (Table 3.2-15), which may indicate that vine growth can be sustained at vine nitrogen levels lower than that required to provide YAN levels sufficient for yeast fermentation requirements. Guidelines for optimal leaf nitrogen concentration to provide YAN concentrations above 140 mg/L may need further research for this variety.

Correction of low YAN levels using DAP in the winery is common. However, if YAN is not measured prior to addition, problems can result from not adding enough, or adding excessive nitrogen (Bell and Henschke, 2005). The relative consistency between vigour

zones across all three seasons in block A suggests that knowledge of the vine vigour level in a block may inform these calculations. While the large changes resulting from seasonal difference indicate that accurate analysis is still important, these results indicate that an area that produced fruit with a high YAN in one season is unlikely to require winery nitrogen supplementation in other seasons. Similarly, an area that produces low-nitrogen must or juice may be targeted for analysis in subsequent seasons.

Grape phenolic concentration was decreased in the highest vigour zone in block A. Increasing vigour zones had a higher canopy density (Table 3.2-11). Decreases in canopy density will lead to increased light interception and higher berry temperature (Smart, 1988). Flavan-3-ols, which condense to make tannins (Adams, 2006), are used by plants to absorb UV light, protecting underlying tissues (Burchard et al., 2000, Downey et al., 2006). While excessive sunlight will in some cases reduce levels, excessive shading will generally decrease phenolic content of fruit, and in particular of the skin (Bergqvist et al., 2001, Joscelyne et al., 2007, Keller and Hradzina, 1998, Ristic et al., 2007, Burchard et al., 2000, Downey et al., 2006). Spayd et al, (2002) demonstrated that the UV portion of light was the most important at stimulating phenolic production, and also that there was no temperature effect. Phenolic compounds may also be decreased by higher nitrogen availability, which was observed in the high vigour musts indicating a high level within the vine, while water deficits will tend to increase grape skin phenolic content (Roby et al., 2004).

Grape anthocyanin concentration did not change across the vigour zones. Anthocyanins will vary in response to sunlight and to berry temperature, with increases in temperature up to 25°C increasing anthocyanin production (Deis et al., 2009), although production will eventually decrease in situations of excessive exposure (Bergqvist et al., 2001), particularly as a result of temperature above 35°C (Spayd et al., 2002). Other studies on vine vigour and anthocyanin content have found that the total amount present in grapes may not be greatly altered by changing vigour levels, although the proportions of the different anthocyanins will shift (Cortell et al., 2007b). Anthocyanin composition was not examined in this trial.

Increased vigour is often associated with increased levels of botrytis (English et al., 1989, Smart, 1991, Thomas et al., 1988), however the results from this trial indicate that the relationship is not necessarily that simple. While vigour, as measured by PCD, did show the strongest correlation with botrytis in block B, PCD was only significantly correlated to botrytis levels in one year out of three in block A. In the first year, the most significant correlations were with fruit yield and average bunch weight. Botrytis growth may be promoted by a number of factors. These include the presence of inoculum, the levels of latent infection from early in the season, appropriate weather conditions late in the season, and control actions made by vineyard managers (English et al., 1989).

3.3e Conclusion

The wide variation in relationship between yield and vigour that was evident indicates that vigour alone should not be used as a proxy measure for yield, as has been suggested by some authors (Hall et al., 2002). While this might limit the applicability of technology such as aerial remote sensing in predicting yields, site-specific correlation of vigour and yield relationship may be possible. This has potential to enhance accuracy of yield estimation techniques.

The changes in berry phenolics evident in this trial, as well as the changes in grape juice acids and yeast assimilable nitrogen concentration, suggests that grapes from different vigour zones will produce different wine styles, which will be further explored in the

following chapter.

This information may be of use in determining treatment of fruit in the winery, following collection of information on the extent and variation of vigour level within a vineyard management unit.

3.4 The impact of variable vine vigour on wine composition and fermentation dynamics in grapevine (*Vitis vinifera* L.) cv Pinot Noir

3.4a Introduction

Despite uniform vineyard management, there can be a large range of vine vigour levels (Bramley and Lamb, 2003). This may lead to variation in fruit and wine attributes (Bramley, 2005, Cortell et al., 2008).

Excessive vegetative growth will decrease the exposure of fruit to light (Smart, 1985). A number of compounds important to winemaking are altered either directly by radiation, or by changing temperatures. Under the normal conditions in a cool climate vineyard, increased exposure to UV light will increase the production of phenolic compounds such as flavan-3-ols (Cortell and Kennedy, 2006, Keller et al., 2003, Spayd et al., 2002)in the grape skin. Flavan-3-ols and tannins are very important to red wine quality, being a major component determining wine astringency (Gawel, 1998) and binding to anthocyanins to stabilise colour in red wines (Fulcrand et al., 2006, Somers and Evans, 1977). Grape anthocyanins are also affected by sunlight exposure, being particularly influenced by grape temperatures (Spayd et al., 2002, Bergqvist et al., 2001, Deis et al., 2009, Ristic et al., 2007). Increasing temperatures from 15°C to 25°C (Deis et al., 2009) can lead to an increase in anthocyanins, with a maximum production around 30°C (Spayd et al., 2002). Berry temperatures above 35° will decrease anthocyanin production, which may occur on sun-exposed fruit in warm climates (Bergqvist et al., 2001, Spayd et al., 2002). Anthocyanins form the basis of colour in red wines, as free anthocyanin in young wines, and bound either by polymerisation to compounds such as tannins, or by co-pigmentation in older wines (Cheynier et al., 2006, Gao et al., 1997, Romero-Cascales et al., 2005). Anthocyanin concentration and colour density has been positively correlated to wine quality (Jackson et al., 1978).

The fermentation process has a major impact on the odour of wine, forming the fermentation bouquet. Yeast assimilable nitrogen (YAN) levels within the ferment will have a major influence on fermentation dynamics, and can influence this bouquet. Decreasing shoot vigour is associated with decreased YAN concentration (section 3.3c, p. 67). Yeast in a must with a nitrogen deficiency can produce excess hydrogen sulphide (Gardner et al., 2002, Hallinan et al., 1999, Jiranek et al., 1995b) that can negatively impact wine quality. These wines may be at risk of slow or sluggish fermentations (Bell and Henschke, 2005, Chaney et al., 2006, Gardner et al., 2002, Varela et al., 2004). Yeast metabolism of nitrogenous compounds will also lead to the production of esters, which can contribute significantly to wine aroma (Bell et al., 1979, Guitart et al., 1999, Henschke and Jiranek, 1993).

An understanding of the impact of different levels of vine growth on wine quality may assist in producing wines to a desired quality specification, and may help in directing vineyard management operations. This chapter details the final stage of a series of investigations in sites with a range of vine vigour levels. Previous chapters have described the canopy differences in detail, and the impact vigour had on fruit attributes and yield.

Fruit harvested from Pinot Noir vines from different levels of vigour was fermented into wine. This study undertook to investigate how vigour was altering the winemaking process and the final wine produced. Combined with a comprehensive understanding of

the canopy attributes of the vine outlined in the previous chapters, this knowledge will increase our understanding of the linkages between soil, vine, fruit and wine.

3.4b Materials and Methods

Trial vineyard description

Trial layout is described in the general materials and methods section (p. 17). Wine was made from block A Pinot Noir in 2005-06, 2006-07 and 2007-08, while wines were made from block B Pinot Noir in 2005-06.

Materials and methods used in these trials are outlined in Table 3.4-1.

*Table 3.4-1 Materials and methods used in trials described in the following chapter. * - more information on this analysis below the table*

Trial	*Analysis*	*Date*	*Reference page*
Block A Pinot Noir	Wine produced and ferment monitored	2006 2007 2008	35
	Somers analysis	05/06* 09/07 07/08	37
	Tannin analysis	07/08*	37
	MS-e_nose	07/08	38
	Sensory analysis*	06/07/09	
Block B Pinot Noir	Wine produced and ferment monitored	2006	35
	Somers analysis	07/2008*	37
	Tannin analysis	07/2008*	37

Winemaking

Winemaking followed the procedures outlined in the general materials and methods (p. 35). The punch-down cap management system was used in all seasons.

Fermentation rates were compared between vigour zones by looking at the Baumé decrease at a point mid-way through the fermentation. This stage was reached 48 hours post inoculation in 2006, 56 hours in 2007 and 70 hours in 2008.

Somers analysis

Wines from block A made in 2006 were assessed at twelve months of age for colour density and hue, buffered for pH and alcohol content variation.

These wines were re-measured using the full Somers analysis in 2008, along with wines from block B. They were two years old by this stage.

Wines made in 2007 and in 2008 were assessed when they were 3 months old.

Tannin analysis

Wines from 2005-06 were measured in 2008.

Mass Spectrometry – e_nose analysis

Mass Spectrometry – e_nose (MS e_nose) analysis followed the procedures outlined in the materials and methods section. Wines made from block A in 2008 underwent MS-e_nose analysis.

Sensory analysis

Wine sensory analysis was carried out on wines from block A Pinot Noir made in 2008, on wines that were sterile filtered and aged for 12 months.

Sensory analysis structure was set up with advice from the AWRI sensory team (L. Francis, *pers.comm.*). Ten tasters were used, all of whom had some level of wine knowledge, and included wine writers, winemakers and enthusiastic amateurs. A sample of wines from across the vigour range was tasted initially, and an open discussion following this was used to generate a list of descriptors.

Tasting sheets were constructed that utilised the list of descriptors (see Appendix). These were judged by getting the tasters to place a mark along an ungraduated 100 mm line. Measuring the point where the taster's mark crossed the line gave the score. Scores were adjusted by calculating the overall range of scores given by each individual judge, and adjusting the score so that their minimum score was equivalent to 0 and their maximum equivalent to 100, and all other scores were proportionately sited within that range. Wines were also scored with the 20 point system commonly known as the Australian wine show system, where appearance is scored out of 3, nose out of 7 and mouth feel out of 10.

The final list of descriptors was:

- Vegetal
- Herbaceous
- Red fruit
- Dark fruit
- Overall fruit (aroma)
- Overall fruit (palate)
- Floral
- Earthy
- Spicy
- Reduced
- Tannins – Smooth to coarse
- Tannins - Quantity

All wines were decanted at least one hour prior to pouring.

Wines were poured in flights of six, with approximately 30 mL into XL-5 wine tasting glasses. Each flight contained two wines that were repeated within that flight.

Following judging, tasters were sorted on the basis of repeatability. The difference in score for each attribute of the repeated samples was compared to the overall range for that taster and that flight. These differences were then average across all repeated samples. Three tasters' results were removed from further analysis due to a low degree of repeatability.

Results were analysed by ANOVA for each descriptor, and by principal component analysis.

3.4c Results

Fermentation rates

Fermentation rates increased with vine vigour, although there were no significant differences between the two lower vigour zones in block A in any season (Figure 3.4-1, Figure 3.4-2, Figure 3.4-3). YAN levels also rose with vigour (Figure 3.3-3) and fermentation rates in block A were strongly linked to yeast assimilable nitrogen levels, with R^2 values of 0.98 in 2006, 0.93 in 2007 and 0.84 in 2008.

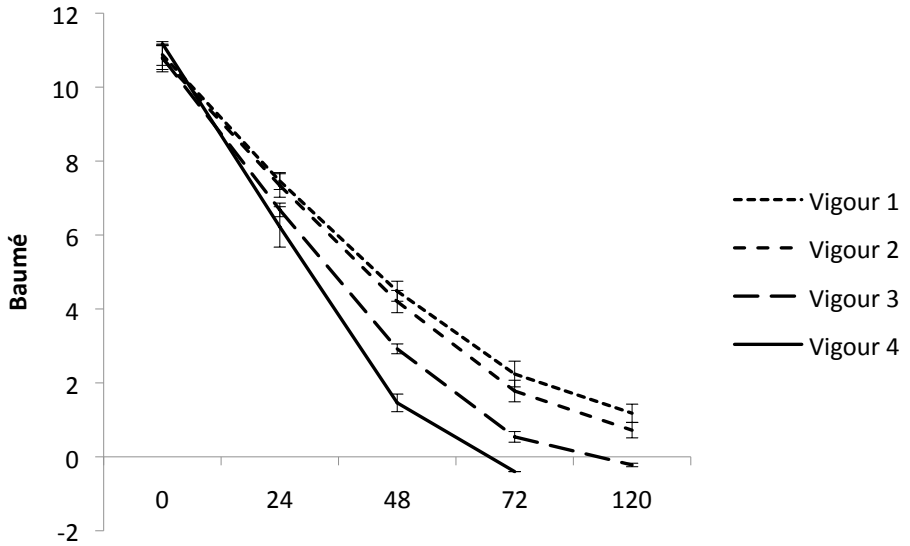

Figure 3.4-1 Ferment progression for each vigour zone in 2005-06 in block A from inoculation to pressing. All wines proceeded to completion following pressing. Error bars represent standard error of the mean.

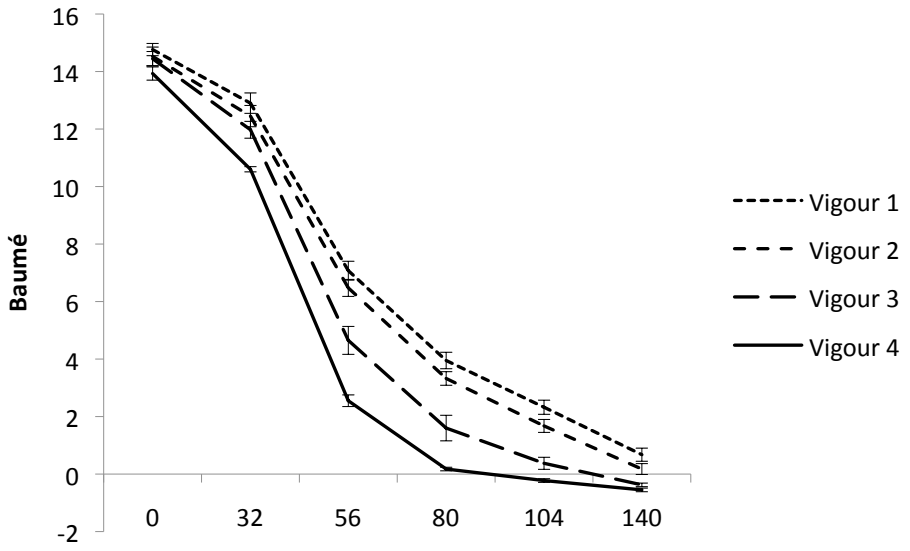

Figure 3.4-2 Fermentation progress for each vigour zone in 2006-07 in block A from inoculation to pressing. All wines proceeded to completion following pressing. Error bars represent standard error of the mean.

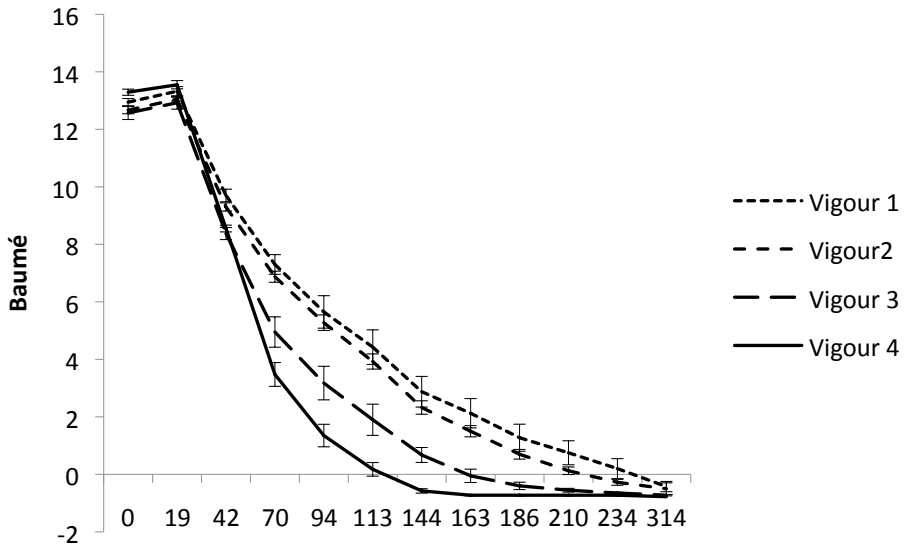

Figure 3.4-3 Fermentation progression for each vigour zone from 2007-08 in block A from inoculation to pressing. All wines proceeded to completion following pressing. Error bars represent standard error of the mean.

Somers analysis

Wines made in block A in 2006 displayed decreasing colour density and increasing hue as vigour increased (Table 3.4-2). Colour density, hue and sulphur resistant pigment levels were all highly significantly affected by vigour level in block A wines from 2006. Chemical age one and two, and anthocyanin ionization are also all reduced by vigour level. Anthocyanin concentration was not significantly impacted, however there is a trend towards increasing levels of anthocyanin with increasing vigour.

*Table 3.4-2 Somers analysis assessed in 2008 for block A wines from the 2005-06 vintage. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Colour Density	Colour Density SO_2 corrected	Hue	Chem age 1	Chem age 2	Anthocyanin (mg/L)	Anthocyanin ionization (%)	SO_2 resistant pigments	Total pigments
1	6.77c	6.64b	0.73a	0.57b	0.35b	52.9	69.7c	2.19b	6.29
2	6.01b	6.06b	0.73a	0.58b	0.35b	53.3	64.9bc	2.04b	6.07
3	5.00a	5.10a	0.78a	0.51ab	0.26a	66.4	43.0ab	1.48a	5.78
4	4.52a	4.60a	0.87b	0.48a	0.21a	78.6	32.8a	1.19a	5.90
Sig	***	***	***	*	*	n.s.	*	***	n.s.

Wines from block B made in 2006 had significant changes in spectral characteristics when measured in 2008 (Table 3.4-3). Colour density decreased with increasing vigour, as did SO_2 resistant pigments, chemical ages 1 and 2, and anthocyanin ionization. Anthocyanin concentration increased as vigour increased, as did hue (Table 3.4-3).

*Table 3.4-3 Somers analysis of wines from block B. Wines were made in the 2005-06 season, and analysed in 2008. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Colour Density	Colour Density SO_2 corrected	Hue	Chem age 1	Chem age 2	Anthocyanin (mg/L)	Anthocyanin ionization (%)	SO_2 resistant pigments	Total pigments
1	5.29c	5.35c	0.84a	0.61d	0.40c	29.5a	75.9d	1.79c	4.5
2	4.22b	4.47b	0.87ab	0.57cd	0.32bc	41.8a	47.3c	1.42b	4.5
3	3.06a	3.42a	0.88abc	0.50bc	0.26b	44.3a	36.1bc	0.93a	3.8
4	2.50a	2.94a	0.91bc	0.43ab	0.15a	69.2b	18.1a	0.70a	4.6
5	2.46a	2.85a	0.94c	0.38a	0.13a	69.5b	19.6ab	0.60a	4.5
Sig	***	***	*	***	***	**	***	***	n.s.

Wines from block A in 2006-07 (Table 3.4-4) did not show the same differences as seen in the wines from the preceding season (Table 3.4-2). Colour density decreased as vigour increases, but this is not significant when SO_2 is corrected for. There are more sulphur-resistant pigments in wines from the lowest vigour zone (Table 3.4-4).

The hue values for the wines in this trial are all significantly higher than the wines from the preceding season, notwithstanding the extra year of age on the 2006 wines.

*Table 3.4-4 Somers analysis of block A wines from the 2006-07 season assessed in 2008. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Colour Density	Colour Density SO_2 corrected	Hue	Chem age 1	Chem age 2	Anthocyanin (mg/L)	Anthocyanin ionization (%)	SO_2 resistant pigments	Total pigments
1	4.88b	5.45	0.81	0.45	0.16	128.7	20.6	1.39b	8.75
2	4.11ab	4.67	0.83	0.41	0.13	125.9	18.5	1.09a	8.11
3	3.64a	4.64	0.84	0.37	0.12	130.5	15.2	0.99a	8.17
4	3.82a	4.44	0.86	0.40	0.11	144.7	14.6	0.99a	8.89
Sig	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

Wines from the 2007-08 season of the trial showed no significant changes in colour density in response to vigour level, although there is a trend towards this (Table 3.4-5). SO_2 resistant pigments were higher in the lower vigour zone wines.

*Table 3.4-5 Somers analysis of block A wines from the 2007-08 season assessed in 2008. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	Colour Density	Colour Density SO2 corrected	Hue	Chem age 1	Chem age 2	Anthocyanin (mg/L)	Anthocyanin ionization (%)	SO2 resistant pigments	Total pigments
1	4.55	5.30	0.235	0.073b	0.67	186.6	21.1b	0.77c	10.61
2	4.12	4.94	0.231	0.073b	0.65	170.3	20.9b	0.71bc	9.70
3	3.67	4.27	0.185	0.050a	0.66	182.9	18.9ab	0.49a	9.96
4	3.90	4.71	0.185	0.047a	0.66	214.7	16.8a	0.54ab	11.63
Sig	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	n.s.

In the 2008 analysis of wines made in 2006, SO_2 resistant pigments were correlated to colour density with an R^2 of 0.94 in block A and 0.97 in block B. This correlation was also significant in block A in 2007 ($R^2 = 0.81$) and 2008 wines ($R^2 = 0.46$). Tannin concentration was significantly correlated to SO_2 resistant pigments in 2006 wines ($R^2 = 0.74$ in block A and 0.79 in block B) and in 2007 ($R^2 = 0.52$) and 2008 ($R^2 = 0.59$) wines from block A. There was no significant impact from anthocyanin concentration on wine colour in either 2006 wines or 2007 wines from block A, however there was a correlation in block B in 2006 ($R^2 = 0.33$) and in block A in 2008 ($R^2 = 0.39$).

Tannin and Phenolics Assessment

The phenolics levels on all wines were significantly impacted by vigour level, in all years. This was clear from the results of the Dambergs tannin assessments (Figure 3.4-4).

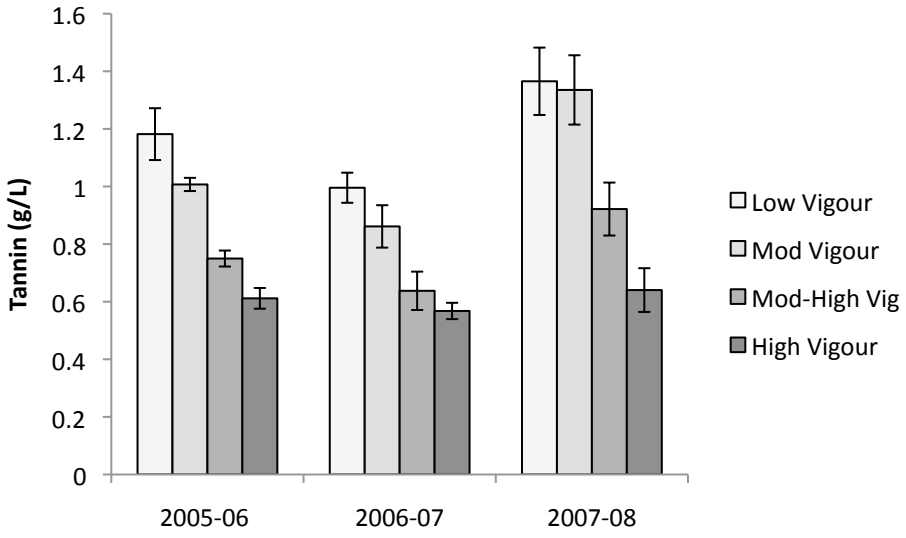

Figure 3.4-4 Tannin concentrations of wine from block A Pinot Noir over three seasons. All wines were sourced from the same sites in each season. Error bars represent standard error of the mean.

Phenolics analysis in block B also indicated that tannins and overall phenolic concentration decreased as vigour increased (Table 3.4-6).

*Table 3.4-6 Phenolics and tannin analysis of wines from 2005-06 off block B, analysed in 2008. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour	*Somers Phenolics*	*Tannin (mg/L)*
1	35.0c	1.45c
2	32.2c	1.24bc
3	27.6b	1.05b
4	24.8ab	0.66a
5	22.6a	0.54a
Sig	***	***

Wine tannin concentration was correlated to vine vigour level in both block A and block B wines made in 2005-06. The relationship in each block between tannin concentration and PCD was very similar (Figure 3.4-5).

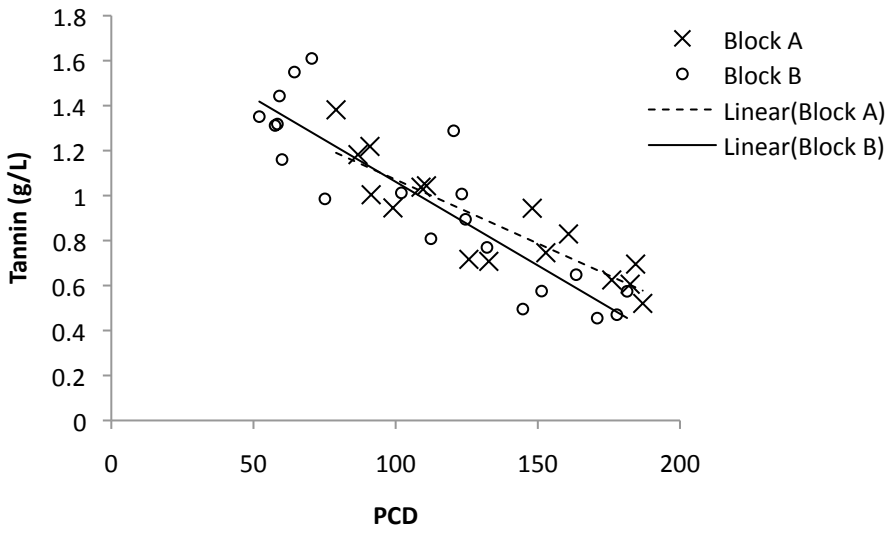

Figure 3.4-5 Wine tannin concentration against plant cell density (per panel average) for block A and block B in wines made in 2005-06. Both blocks are the same Pinot Noir clone, trellised to a Scott Henry trellis. R^2 for block A = 0.78; R^2 for block B = 0.79.

Wine E-nose analysis

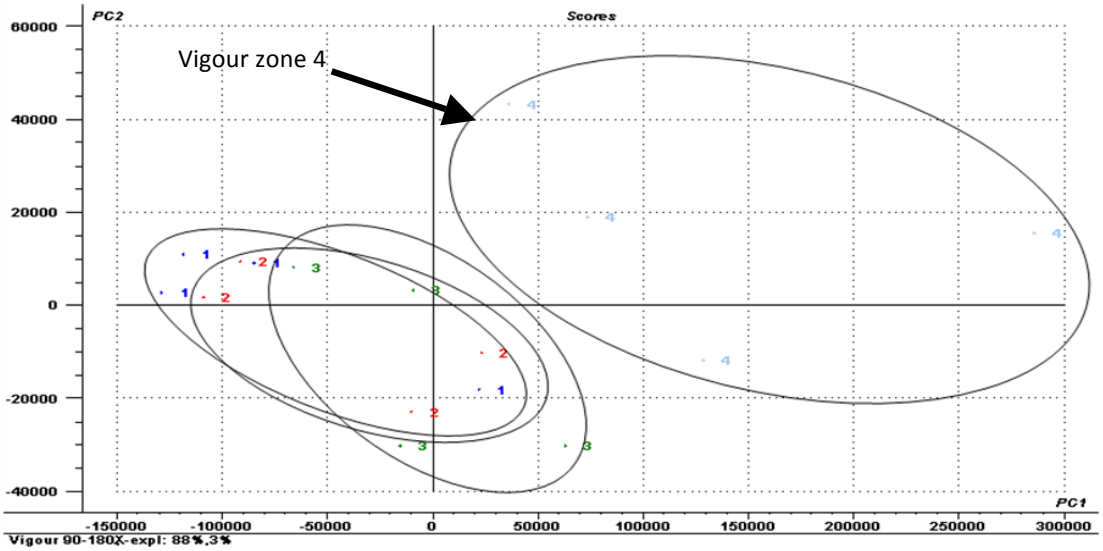

Figure 3.4-6 Principal component analysis output of MS-e_nose data from block A in 2007-08. 88%of the variability is explained by the X-axis values, while 3% of the variation is explained by the Y-axis values.

E-Nose analysis of wines from the 2007-08 harvest indicated that there were major sensory differences between the highest vigour zone and the other three vigour zones, however there was not a large difference between the first three vigour zones (Figure 4.4-6).

PCA analysis of the sensory data indicated that there were significant groupings for the different vigour zones (Figure 3.4-7).

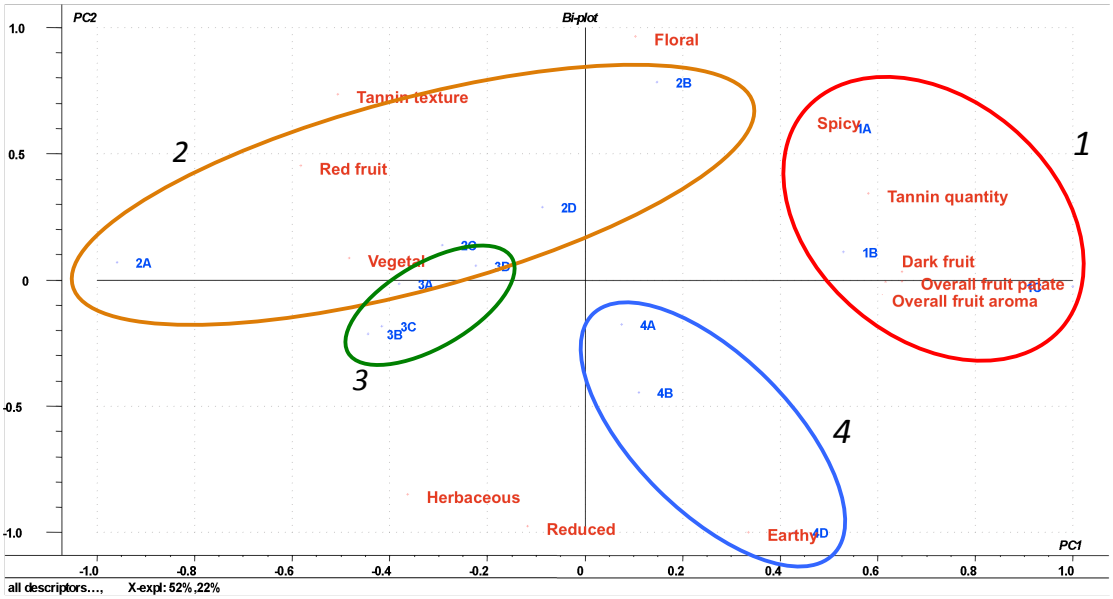

Figure 3.4-7 Principal component analysis output of sensory analysis of wines from block A Pinot Noir in the 2007-08 season. Best 7 tasters, two outlier points removed. Numbers represent the vigour zones (1 = low, 4 = high), and letters indicate the replicate block for each vigour zone. 52% of the variability is explained by PC1 (X-axis), and 22% of the variability is explained by PC2 (Y-axis).

ANOVA analysis indicated that there were significant differences between a number of the wine attributes (Table 3.4-7, Table 3.4-8). Wines from low vigour vines scored more highly for dark fruit characteristics but lower for red fruit character, and scored higher for fruit on the palate. Wines from the lower vigour zone vines had a lower level of herbaceous character, and there was a non-significant trend towards the lowest vigour wine being less vegetal. The tasters observed the higher tannin concentration of the lowest vigour zone wine. The sensory analysis tannin quantity scores of wines from vigour zones 2 and 3 were not significantly different to the wines from vigour zone 4, and the palate score also decreased with increasing vigour. Tannin coarseness measures did not correlate with tannin quantity measures, with the second lowest vigour zone wines scoring highest for this attribute, while zone four wines had the lowest.

Wines from the highest vigour vines were observed as having a significantly higher earthy characteristic.

*Table 3.4-7 Scores for overall wine sensory assessment, based on the 20-point scoring system, for wines from four vigour zones (zone 1 = low vigour; zone 4 = high vigour) (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	*Appearance score*	*Aroma score*	*Palate score*	*20 point score*
1	2.9	4.9	7.3b	15.1
2	2.9	4.7	7.0ab	14.7
3	2.9	4.7	6.7a	14.3
4	2.8	4.9	6.9a	14.4
Sig	n.s.	n.s.	**	n.s.

*Table 3.4-8 Scores for specific wine attributes from wines made from four vigour zones (zone 1 = low vigour; zone 4 = high vigour) (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Vigour zone	*Vegetal*	*Herbaceous*	*Red Fruit*	*Dark fruit*	*Overall fruit aroma*	*Overall fruit palate*	*Floral*	*Earthy*	*Spicy*	*Reduced*	*Tannin coarseness*	*Tannin quantity*
1	22	25a	42a	58b	55	60b	33	25a	38	12	29ab	54b
2	28	30ab	55b	41a	48	49a	34	19a	37	13	36b	45a
3	29	34bc	52b	41a	48	50a	29	22a	29	11	32ab	42a
4	30	38c	49ab	46a	53	53a	32	34b	33	17	26a	42a
Sig	n.s.	*	*	***	n.s.	*	n.s.	**	n.s.	n.s.	*	**

3.4d Discussion

There was a significant difference in the fermentation rate between vigour zones, with the low vigour zones fermenting more slowly than the high vigour zones. These rates were correlated to the must YAN concentration, which rose with vigour (Figure 3.3-3). Increases in must YAN levels up to 500 mg/L in the must have been reported to lead to

increases in fermentation rate (Bisson, 1991). The maximum YAN levels in this trial were below this level, therefore the continued rise in fermentation rate was not unexpected.

Yeast dynamics during fermentation can have an impact on wine attributes. Yeast stressed by low nutrient levels can produce hydrogen sulphide (Gardner et al., 2002, Hallinan et al., 1999, Jiranek et al., 1995b, Sponholz, 1991), leading to reductive odours (Bell and Henschke, 2005). Sensory analysis of the wines did not indicate that there was any increase in perception of reductive aromas in the low vigour zone wines, indicating that the low YAN concentration of these wines was not sufficiently low to create excessive hydrogen sulphide. The PCA plot indicated that the group displaying the greatest degree of reductive aroma was the high vigour zone wine, although whether this is due to higher hydrogen sulphide levels was not assessed (Figure 3.4-7). YAN levels in the fruit in this trial ranged from an average around 130 mg N/L in the low vigour zones, up to over 250 mg/L in the high vigour zones. The minimum YAN level to avoid fermentation stress recommended in the literature is 140 mg/L (Butzke, 1998, Bely et al., 1991), however this may be modified by the yeast strain (Jiranek et al., 1995b), and also by the release of nitrogen compounds from grape skin and seeds, which will not be included in analysis of the juice alone, as was performed in this trial.

Faster fermentation times are not always an advantage. A fermentation that proceeds too quickly can lead to excess production of heat, which may stress yeast (Jackson, 1994), and potentially a reduced extraction period if wines are pressed off skins sooner than otherwise (Bell and Henschke, 2005). Although this was minimised by the size of the ferments used in this study, large commercial ferments are more prone to excess heat.

YAN rises with increasing vigour were noted to be stable over the three years of the study in block A Pinot Noir. Grapes from high vigour areas therefore may be earmarked as potentially needing more cooling during fermentation, or other pre-emptive steps to accommodate the higher YAN levels, and conversely grapes that have low YAN may be prioritised for YAN assessment in following seasons, on the basis of historical performance. Conversely, wines from lower vigour zones may have a consistent requirement for the use of DAP to adjust YAN levels.

Vine vigour had a range of impacts on colour and colour components, however this effect was not consistent in every season. Wines made from vigour zones 1 and 2 in both block A and block B in 2006 had a higher colour density than the wine from the higher vigour vines (Table 3.4-2, Table 3.4-3). In 2007 this difference was less apparent (Table 3.4-4) although low vigour wines still had a significantly higher colour density than the other vigour zones on non-SO_2 adjusted wines. However, in 2008 there were no significant differences in wine colour density (Table 3.4-5). Other studies that examined the impact of vigour on colour have found that increasing vine vigour or fruit shading can decrease colour density of wines (Cortell et al., 2007a, Joscelyne et al., 2007, Mazza et al., 1999). Cortell et al. (2007a) and Gao et al. (1997) note that the changes in the amount of pigmented polymers present in Pinot Noir wines were a major determinant of colour density in the wines. Cortell et al. (2007a) also reported that the quantity of pigmented polymers was significantly correlated to the total proanthocyanidin concentration of both the fruit and the wine. Although proanthocyanidins were not specifically measured in this study, this group of compounds form a major part of the total wine phenolic concentration (Kennedy et al., 2006), which did decrease as vigour increased in all trials. Pigmented polymers will contribute to the Somers SO_2-resistant pigment measurement, which was also seen to decrease with higher vigour in all years in this trial. Regression analyses indicated that the SO_2 resistant pigment concentration of the wines was very important in determining wine colour density. This correlation increased with the age of the wine when the majority of anthocyanins would have been

incorporated into pigmented polymers (Bakker et al., 1998, Gao et al., 1997, Somers and Evans, 1977). Medina et al. (2005) found a similar result, with a strong correlation between wine colour and anthocyanin-derived compounds, but no correlation to free anthocyanin levels.

The wine phenolics and tannins of wines from low vigour vines were greater than in wines from higher vigour zones in all years of the trial. Grapevines are stimulated to increase the production of proanthocyanidins and flavan-3-ols when exposed to UV light. Studies of fruit exposure effects have indicated that excess shading will lead to a decrease in phenolic compounds in the fruit (Bergqvist et al., 2001, Cortell and Kennedy, 2006, Joscelyne et al., 2007, Ristic et al., 2007, Rustioni et al., 2009, Spayd et al., 2002) and the resulting wines (Cortell and Kennedy, 2006, Joscelyne et al., 2007, Mazza et al., 1999, Ristic et al., 2007). Increased canopy density will increase shading (Smart, 1985, Smart, 1988), and studies that have linked vine vigour to wine phenolic content have seen that increasing vigour decreased the proanthocyanidins in the wine (Cortell et al., 2005, Cortell et al., 2007a, Proffitt et al., 2006).

The strength of this relationship in these trials indicates that measures of vigour, such as infrared imagery or pruning weight measurement, may be directly correlated to the wine tannin concentration. The strong correlation between plant cell density (PCD) and wine tannin concentration in wines from both block A and block B (Figure 3.4-5) are one example of this, and are similar to results achieved by Lamb et al. (2004). Canopy density measured by point quadrat at veraison in 2007 (Table 3.2-11) indicated that the increasing vigour between vigour zones 3 and 4 was seen as an increase in density in the upper section of the canopy, with no increase in leaf layer number in the fruit zone. This may have implications for management, particularly when leaf removal is undertaken to increase fruit tannins, since that traditionally occurs only in the fruit zone. It is possible that leaf layers prior to veraison are denser in vigour zone 4 than vigour zone 3, which is the period when flavan-3-ols and proanthocyanidins are manufactured in the berry (Harbertson et al., 2002).

Other Somers analyses that differed between vigour zones included a decrease in chemical age 1 and 2 in the older 2006 wines, and chemical age 2 in 2008 wines, with increasing vigour. Both measures will increase along with the amount of pigmented polymers present, and will therefore be changing in response to changes in phenolic compound concentration. The decreased hue values of wines from lower vigour vines also indicates that these wines are less oxidised than the wines from the highest vigour zone (Table 3.4-2). Hue values increase with more oxidation of the wine (Somers and Evans, 1977). The changes in hue are likely to be more apparent in these wines because of the increased wine age. Anthocyanins are made more resistant to oxidation when bound to tannins (Jackson, 1994, Ribéreau-Gayon and Glories, 1986), therefore an increase in tannins is most likely responsible for the lower hue value in the low vigour wines.

Anthocyanins can exist in a range of forms, of which only the flavilium ion form is coloured (Jackson, 1994). Flavilium ions represent around 20% of the total free anthocyanin concentration, however when anthocyanins are condensed together with tannins, a greater proportion are in a form that is coloured (Ribéreau-Gayon and Glories, 1986). This is measured in Somers and Evans' (1977) anthocyanin ionization analysis. The anthocyanin ionization of the 2006 wines and the 2008 wines decreased as vigour increased. Anthocyanin ionization will also vary with changes in pH and free SO_2, however pH was held constant and SO_2 additions had been consistent between treatments. Therefore, the increased tannin level leading to more anthocyanin polymerisation is likely to have been responsible for this result.

The level of anthocyanin concentration in the wines from the different vigour zones in block A did not vary with vigour in any season they were assessed. Block B wines from 2006 exhibited an increase in anthocyanin concentration with increased vigour. Anthocyanin content in fruit may decrease under shaded conditions (Bergqvist et al., 2001, Ristic et al., 2007), and shading has induced changes in anthocyanin content of wines made using Shiraz grapes (Joscelyne et al., 2007). Studies by Cortell et al. (2007b) however noted that anthocyanin concentration did not change consistently with vigour zone. Spayd et al (2002) noted that light played much less of a role than berry temperature in production of anthocyanins. Cortell and Kennedy (2006) and Ristic et al. (2007) reported that placing light exclusion boxes around Pinot Noir and Shiraz grapes respectively did not significantly alter total anthocyanins, although in both cases it did alter the composition of those anthocyanins with more cyanidin and peonidin glucosides present in the shaded fruit.

Sensory analysis

MS e_nose analysis of the wines indicated that wines from vines in the highest vigour zone had significant sensory differences from the other vigour zones (Figure 3.4-6). The PCA of the spectra did not indicate a major difference between the other three vigour zones.

Sensory analysis of the wines made in 2007-08 provided more detail than the MS e_nose data. The principal component analysis from the sensory assessment of the wines found that there were distinct separations between wines from low vigour vines and those from high vigour vines. Vigour zones 2 and 3 were more closely aligned (Figure 3.4-7). The ANOVA analysis of individual attributes and the PCA indicated that the tasters found the lowest vigour zone vines produced wines that had more dark fruit aroma and overall fruit palate. The tasters identified these characters as being associated with riper fruit, compared to the red fruit flavours. Low vigour vine wines also scored lower for herbaceous character, and there was a trend towards decreasing vegetal score. These all indicate that the lowest vigour zone wines had flavours that would characterise them as "riper" than the higher vigour zone wines. The sugar level of the lowest vigour zones did not vary significantly from any other vigour zone (Table 3.3-11), while pH was not significantly different, and TA was lower (Table 3.3-13). This indicates that the ripeness of the fruit did not differ greatly on the level of these major compounds. Studies on Shiraz have found that increased shading can lead to decreased fruit flavour in the wines (Ristic et al., 2007).

The flavour profile shifted towards attributes associated with less ripe fruit as vigour increased. Herbaceous flavours were linked to the increased canopy density, and leaf growth, as has been found by other researchers (Jackson and Lombard, 1993). The high vigour wines had an increase in earthy flavours. Wines from the two moderate vigour zones were more closely linked to vegetal characteristics, and red fruit characters.

The scores given for tannin quantity also indicate that the tasters were able to detect the higher tannin concentration in the low vigour wines. However, chemical analysis of these wines had found that the tannin concentration in wines from the vigour zone 2 did not differ significantly from vigour zone 1, and that both were significantly higher than the wines made from the highest vigour zone (Figure 3.4-4). Tannin perception can be altered depending on the interactions between acids in the wine (Gawel, 1998), and may also alter depending on the type of tannin. This may explain the discrepancy between the sensory analysis of tannin concentration in the lowest vigour zone and the next highest zone, compared to the Dambergs tannin analysis results. These wines have a similar

tannin concentration, however the low vigour wines were assessed by the tasters as having significantly more tannin. There was no significant difference in either pH or titratable acidity between these wines however.

The changes in individual attributes were not associated with any change in overall wine score, with all wines being similar (data not presented).

Conclusions

Fermentation dynamics and wine quality differ when using grapes from vines of different vigour levels. YAN concentrations increases, which lead to more rapid fermentations, while wine tannin and phenolics decrease.

Improved control over wine quality may be achieved if vine vigour is assessed as part of a vineyard management program. This could allow the fruit from vines of a common vigour level to be harvested separately, or vines managed in a manner appropriate to their vigour level. Aerial remote sensing is one tool with good potential for this purpose, having a strong relationship with tannin concentration in Pinot Noir.

High vigour vines may still produce wine that is perceived as being of a reasonable quality, but they will be significantly different to wine from low vigour vines.

4 Investigations in to altering vine leaf health with nitrogen fertiliser, and impacts on canopy growth, yield, fruit attributes and wine quality in grapevines (*Vitis vinifera* L.) cv Pinot Noir

4.1 Introduction

Vine leaf health can impact wine quality by changing exposure to sunlight, or by acting as sources or sinks for nutrients and carbohydrates. Nitrogen can be a major limiting factor on vine growth, being the element taken up in the greatest quantities from the soil medium, and has well documented impacts on leaf health and canopy growth.

The following chapters detail investigations into how nitrogen applications in the vineyard can impact vines and the resulting wine quality in Pinot Noir table wine. Both changes in nitrogen rate as well as changes in application timing are investigated.

The first chapter details investigations linking nitrogen application with vine canopy growth and leaf health, including how the canopy microclimate is altered and how chlorophyll concentration and leaf senescence respond to nitrogen availability.

The second chapter describes investigations into the impact of nitrogen fertiliser addition on yield, fruit maturation, and fruit chemical attributes, as well as the impact on disease incidence.

The third chapter describes the impacts of the various nitrogen treatments on fermentation dynamics and wine attributes.

Nitrogen may also be added in the winery as diammonium phosphate where levels are below that suitable for yeast growth. The fourth chapter describes comparisons between additions of nitrogen in the vineyard with additions to the ferments, and also investigates the impact of addition at different stages of the ferment.

4.2 The impact of addition of nitrogen fertiliser to grapevine (*Vitis vinifera* L.) cv Pinot Noir at different application timing and rates: Effects on vine canopy and leaf health

4.2a Introduction

Nitrogen deficiency is a major contributor to poor leaf health in vineyards, along with water stress (Keller, 2004). Nitrogen deficiencies are easily remedied by adding nitrogen fertiliser, however the rate of nitrogen is very important, since high rates of nitrogen may stimulate vines to produce excessive growth which can reduce wine quality (Löhnertz, 1991, Smart, 1991). Timing of nitrogen application may also impact the vine's response (Bettiga and West, 1991, Conradie, 1991, Conradie, 2001, Holzapfel and Treeby, 2007, Peacock et al., 1991). In many regions nitrogen applications post harvest are recommended, to coincide with the late-season uptake period and to increase nitrogen storage for the following season (Peacock et al., 1989, Bettiga and West, 1991, Conradie, 2004, Conradie and Saayman, 1989). However, this requires that an active canopy to be present at the time of application to allow nitrogen uptake to occur, which may be an issue in cool climate regions such as Tasmania, where there can often be poor leaf health at harvest (Bettiga and West, 1991, Peacock et al., 1991).

This study was established to examine the impact of nitrogen addition in a cool climate Pinot Noir vineyard with a pre-identified issue of premature leaf senescence. An initial trial into the relative impact of nitrogen availability and irrigation level indicated the importance of nitrogen to leaf health. Over subsequent seasons, vines were fertilised with different rates of nitrogen applied at different times, to manipulate late season vine leaf health levels. The objective was to identify links between nitrogen availability and vine vegetative growth and leaf health, providing a base for further research (detailed in the following chapters) investigating the impact of nitrogen fertiliser on yield and fruit quality characteristics, and wine attributes. The research also aims to have a practical benefit to vineyard operators, to help in determining nitrogen management strategies.

Leaf health was selected as a focal point for the study because it is a visually obvious feature of a vine, which will respond to changes in nitrogen availability. Developing an improved understanding of leaf health links to vine growth and the resulting fruit and wine attributes may allow the use of leaf health as an indicator of overall vine performance. For instance, leaf chlorophyll may have potential use as an indicator of vine nitrogen (Spring and Jelmini, 2002).

4.2b Materials and Methods

Trial Summary

Trials in this chapter include the nitrogen application and irrigation trial conducted in 2005-06 and the nitrogen rate by timing trial, conducted in 2006-07 and 2007-08.

The nitrogen application and irrigation trial consisted of three rates of nitrogen (0, 17 and 51 g N/vine) and three irrigation rates (0, standard and high). Treatments were applied at veraison in the 2005-06 season. Full trial layout is described in the materials and methods section (p. 22).

The nitrogen rate by timing trial consisted in 2006-07 of three nitrogen rate (20, 35 and 50 g N/vine) applied at four dates (pre-bloom, post bloom, pre-veraison and post veraison), and an untreated control. In 2007-08 nitrogen was reapplied to all treatments except the 35 g N/vine treatment, which was also excluded from monitoring. Full trial layout is described in the materials and methods section (p. 22).

*Table 4.2-1 Trial layout summary for investigations into the nitrogen impact on vine vegetative growth. * - more information on this analysis below the table*

Trial	*Analysis*	*Date*	*Reference Page*
Nitrogen and Irrigation Trial	Vigour scorecard	7/04/06	24
	Leaf retention*	20/04/06 12/05/06	
	Chlorophyll concentration estimate	20/02/07	26
	Bud burst %*	15/10/07	
	Early season shoot growth	15/10/07 30/11/07	28
	Periderm development	12/05/06 20/02/07*	29
	Pruning weight	15/06/06 20/07/07	28
Nitrogen rate by timing trial	Leaf counts*	24/01/07 4/04/07 26/04/07 23/04/08*	
	Chlorophyll concentration estimate*	20/02/07 12/12/07 15/01/08 11/02/08 7/03/08 24/03/08 19/04/08	26
	Leaf light transmission	21/03/08	27
	Leaf area	20/03/08	26
	Nitrogen content	27/01/07 04/12/07 12/02/08	29
	Leaf anthocyanin assessment (vein redness)	4/04/07 17/04/08	27
	Bud burst*	14/11/06 12/12/07 14/11/08	
	EL stage and leaf counts*	22/09/07 6/11/07 14/11/08	28
	Shoot tip activity	8/01/07	28
	Periderm browning	6/02/07	29
	Lateral branches and nodes*	23/04/07	
	Shoot length*	14/12/07	
	Pruning weight	20/07/07 4/07/08	28
	Point Quadrat	23/03/08	28
	Canopy light penetration	3/03/08	29

Leaf counts and leaf retention

Visual estimations of the percentage of leaf loss were made in the nitrogen by irrigation trial on the 20th April 2006. Leaf and node counts were made on a number of shoots prior to and at intervals during assessment, to calibrate the assessor's eye.

On the 12th of May 2006 in this trial all leaves remaining on the measurement vines were counted.

In the following seasons (2006-07 and 2007-08) in the nitrogen rate by timing trial, leaf retention was assessed using monitor shoots on the measurement vines that allowed repeated assessment. In the 2006-07 season, leaves were counted on the 24th of January, 4th of April and 26th of April. During the leaf count on the 24th of January main shoot nodes and lateral shoot nodes were also counted. In the 2007-08, leaf and node counts were made on the 23rd of April, 2008, separating leaves and nodes into those on the main shoot and those on lateral shoots.

Leaf retention was calculated by dividing the leaves on the shoot by the nodes on the shoot. Shoot trimming operations were carried out in this vineyard.

Leaf chlorophyll estimates

Leaf chlorophyll concentration was estimated using the method detailed in the general materials and methods section (p. 26). Readings were taken in 2006-07 in the nitrogen timing by rate trial on the 26th of March from randomly selected leaves.

In 2007-08, readings were taken on the monitor shoots on leaves at node positions 4, 6, 9 and 12. If no leaf was present no recording was taken. Readings were taken on the 12th of Dec, 15th of Jan, 11th of Feb, 7th of March and the 24th of March. No readings were taken at node 12 in December, because these leaves were still immature at that date.

These readings were also used to illustrate leaf loss through the season on the main shoot.

Shoot length

Shoot length was measured from the base to the tip.

Node counts

The number of main shoot and lateral nodes were tallied. Lateral shoots were also assessed, to allow a calculation of leaves per lateral shoot.

Bud burst

Bud burst assessments were carried out in 2006-07 on the fertilisation and irrigation trial, and in 2007-08 and 2008-09 on the nitrogen timing by rate trial. Counting cane nodes and shoots early in the season was used to assess bud burst, as a percentage and also as a count of blank nodes and shoots per vine.

Statistical analysis

Refer to the general materials and methods (p. 38).

Abbreviations used in this chapter:

PrB = pre-bloom; PoB = post bloom; PrV = pre-veraison; PoV = post veraison

4.2c Results

Scorecard – Nitrogen by Irrigation Trial

Scorecard assessment indicated that nitrogen addition led to decreased gaps, greener leaves, increased canopy density, decreased fruit exposure and larger leaves retained late in the season (Table 4.2-2). Removing irrigation led to an increased number of gaps, more leaf yellowing, lower canopy density and more fruit exposure. Supplementing irrigation led to larger leaves retained and greener basal leaves, although there was little difference in canopy density and gaps (Table 4.2-3). There were no significant interaction effects.

Table 4.2-2 Scorecard results of vine response to nitrogen application

N rate kg/ha	*Gaps*	*Leaf size*	*Leaf colour*	*Canopy density*	*Fruit exposure*	*Total score*
0	1.9a	2.1a	2.1a	1.6	2.3a	10.0a
50	2.2a	2.3b	2.5b	1.7	3.3b	12.0b
150	2.9b	2.5b	3.2c	1.9	4.0b	14.4c
Sig	***	**	***	n.s.	***	***

*Table 4.2-3 Scorecard results of vine response to irrigation level post veraison. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Irrigation	*Gaps*	*Leaf size*	*Leaf colour*	*Canopy density*	*Fruit exposure*	*Total score*
Nil	1.8a	2.3a	2.2a	1.5a	2.1a	9.8a
Norm	2.5b	2.2a	2.6b	1.9b	3.4b	12.6b
High	2.7b	2.5b	2.9b	1.9b	4.0b	14.0b
Sig	***	*	***	*	***	***

Leaf Health Assessments

Leaf counts and leaf retention

In the nitrogen by irrigation trial, leaf retention was improved by addition of nitrogen. Supplemental irrigation led to increased late season leaf retention late in April, but this was effect was not present in leaf counts three weeks later (Table 4.2-4).

*Table 4.2-4 Late season leaf retention as a result of veraison nitrogen application and irrigation changes in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Rate*	*Leaf loss 20th April (%)*	*Leaf counts per vine May 12th*
N rate g	0	77.5c	0.0a
N/vine	17	43.3b	1.0a
	51	30.8a	4.7b
	Sig	***	*
Irrigation	Nil	57b	2.25
	Norm	53b	1.00
	High	42a	2.42
	Sig	***	n.s.

Pre-bloom nitrogen applications in 2006-07 stimulated a significant increase in leaf counts taken at the end of January in the same season but there were no significant

differences in leaf counts between the controls and vines with post bloom or pre-veraison nitrogen application (Figure 4.2-1). A major proportion of the increase in leaf counts on pre-bloom vines was a result of more lateral leaves (Figure 4.2-2), due to the main shoots being trimmed during normal vineyard management operations.

Late season leaf counts indicated that the early increase in leaf number did not translate into more leaves at the end of the season, compared to other application timings. At the beginning of April, there were no significant differences in leaf counts from any application timing of nitrogen, nor from any of the different rates of nitrogen. By the end of April, the pre-bloom nitrogen application was no longer showing any significant increase in leaf counts (Figure 4.2-1) nor in leaf retention above the control (Figure 4.2-4A). Vines receiving post bloom, pre veraison and post veraison applications were all still retaining significantly more leaves than both the vines that received nitrogen pre-bloom and the control vines.

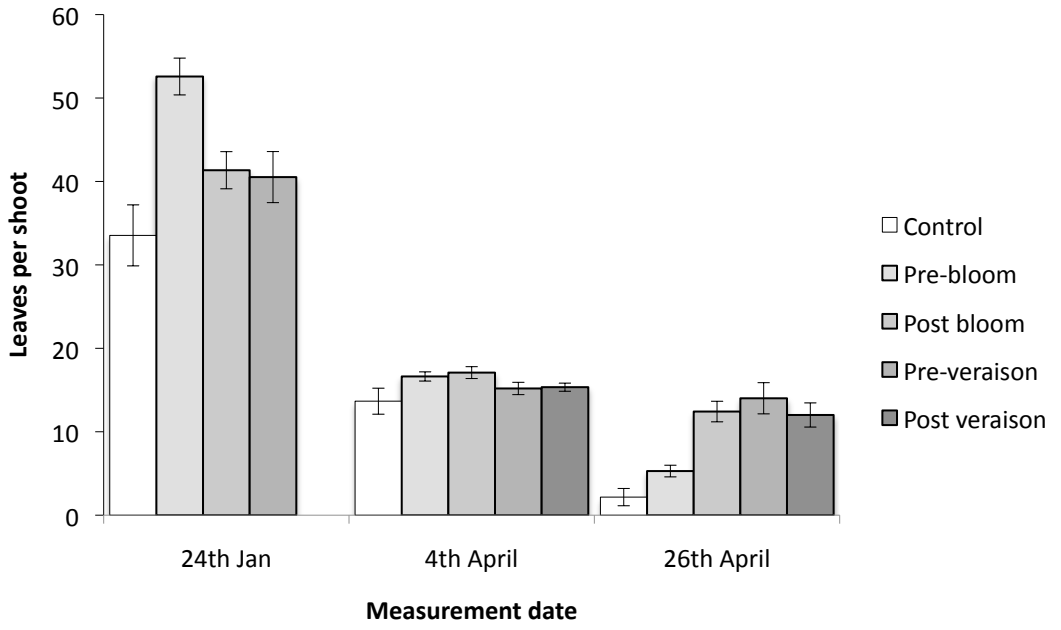

Figure 4.2-1 Response from nitrogen application timing on leaf counts per shoot in 2006-07 (note that the post veraison nitrogen application treatments were not assessed at the end of January). Error bars represent standard error of the mean.

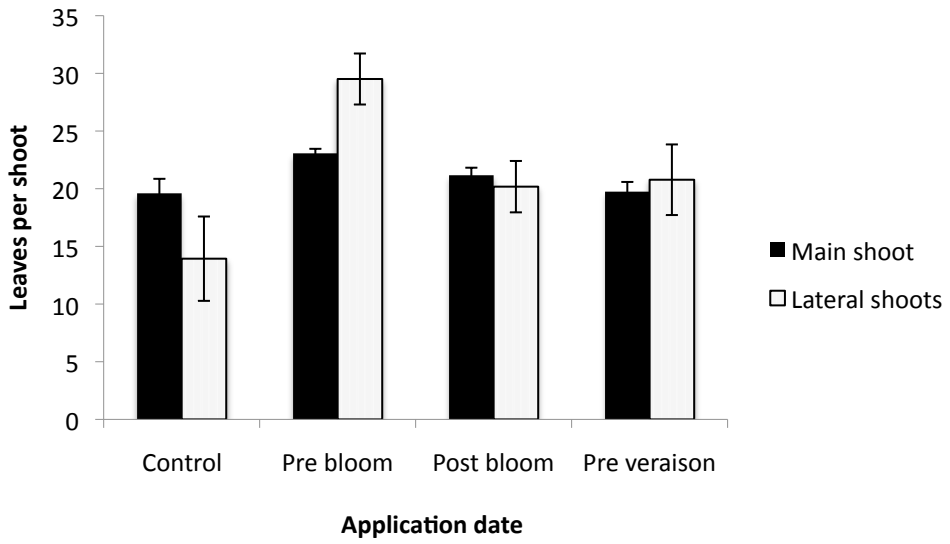

Figure 4.2-2 Leaves per shoot in January 2007 as a result of nitrogen application timing. Error bars represent standard error of the mean.

Nitrogen application led to an increase in leaf counts on the 24th of January, however there was no difference between application rates (Figure 4.2-3). There were no differences as a result of any rate of nitrogen on the 4th of April. The late April assessment of leaf counts was the only measurement where there was an impact from the rate of nitrogen application on leaf numbers and on leaf retention The highest rate of 50 g N/vine led to significantly more leaves per shoot than either 20 or 35 g N/vine. Both of these rates were significantly higher than the control. The leaf retention levels of the vines indicates that the leaf counts on the vines that received 50 g N/vine were being increased by an increase in node number, with retention per node not being significantly different to either the 20 g N/vine or the 35 g N/vine treatments (Figure 4.2-4B). All were greater than the control.

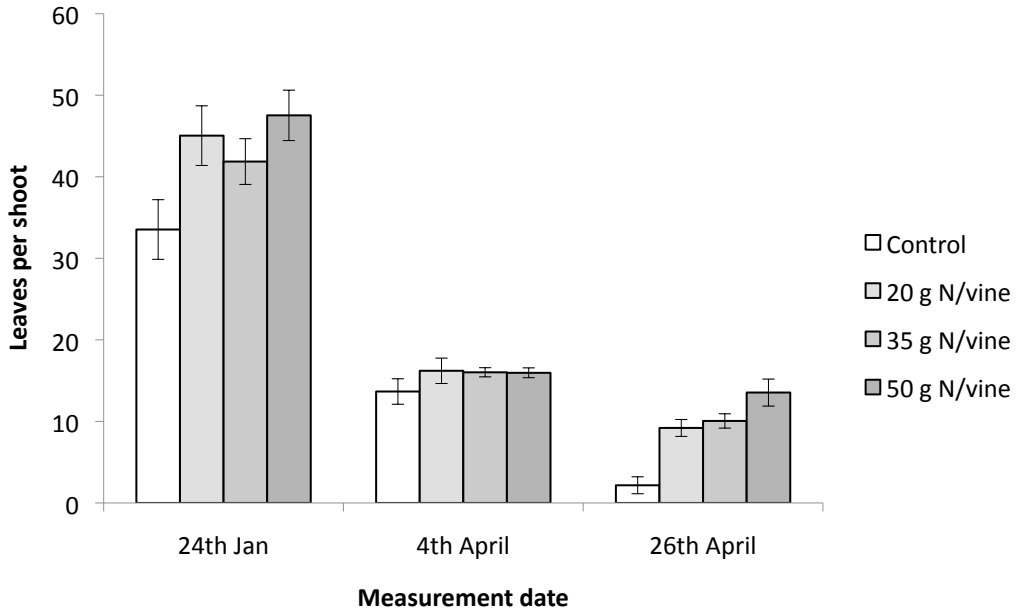

Figure 4.2-3 Leaf counts per shoot of vines treated with 0, 20, 35 and 50 g of nitrogen per vine in the 2006-07 season assessed at three different dates. Error bars represent standard error of the mean.

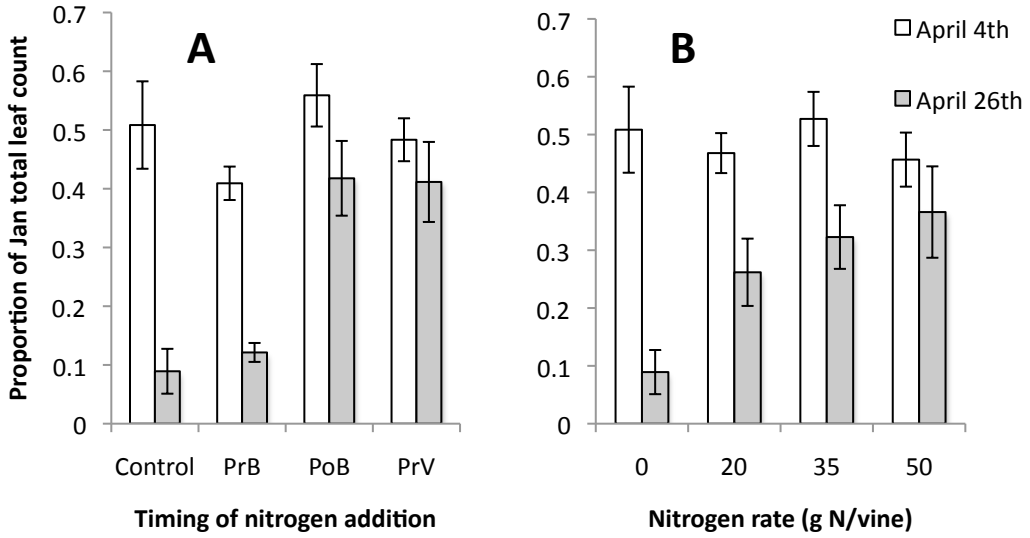

Figure 4.2-4 Leaf retention on the 4th and the 26th of April, expressed as proportions of the January leaf counts, as affected by application timing (A) and rate (B), in 2006-07. Timings were PrB – pre-bloom; PoB – post bloom; PrV – pre-veraision. Error bars represent standard error of the mean.

Nitrogen applications increased leaf retention late in the season in 2007-08 (Figure 4.2-5A, B), although pre-bloom applications were less effective at increasing late season leaf counts than those treatments where nitrogen was applied later in the season (Figure 4.2-5B). The later additions led to more leaves per node (Figure 4.2-5F), but timing did not significantly alter the number of nodes per shoot (Figure 4.2-5E). Increased rate of nitrogen application increased the number of leaves retained (Figure 4.2-5A), however this was not through an increase in leaf counts per node (Figure 4.2-5B), but as a result of more nodes per shoot (Figure 4.2-5C). There were no increases in the number of

nodes on the main shoot in any treatment, as a result of trimming of excessive growth in this block, so all differences in node counts represent impacts on lateral shoot node number.

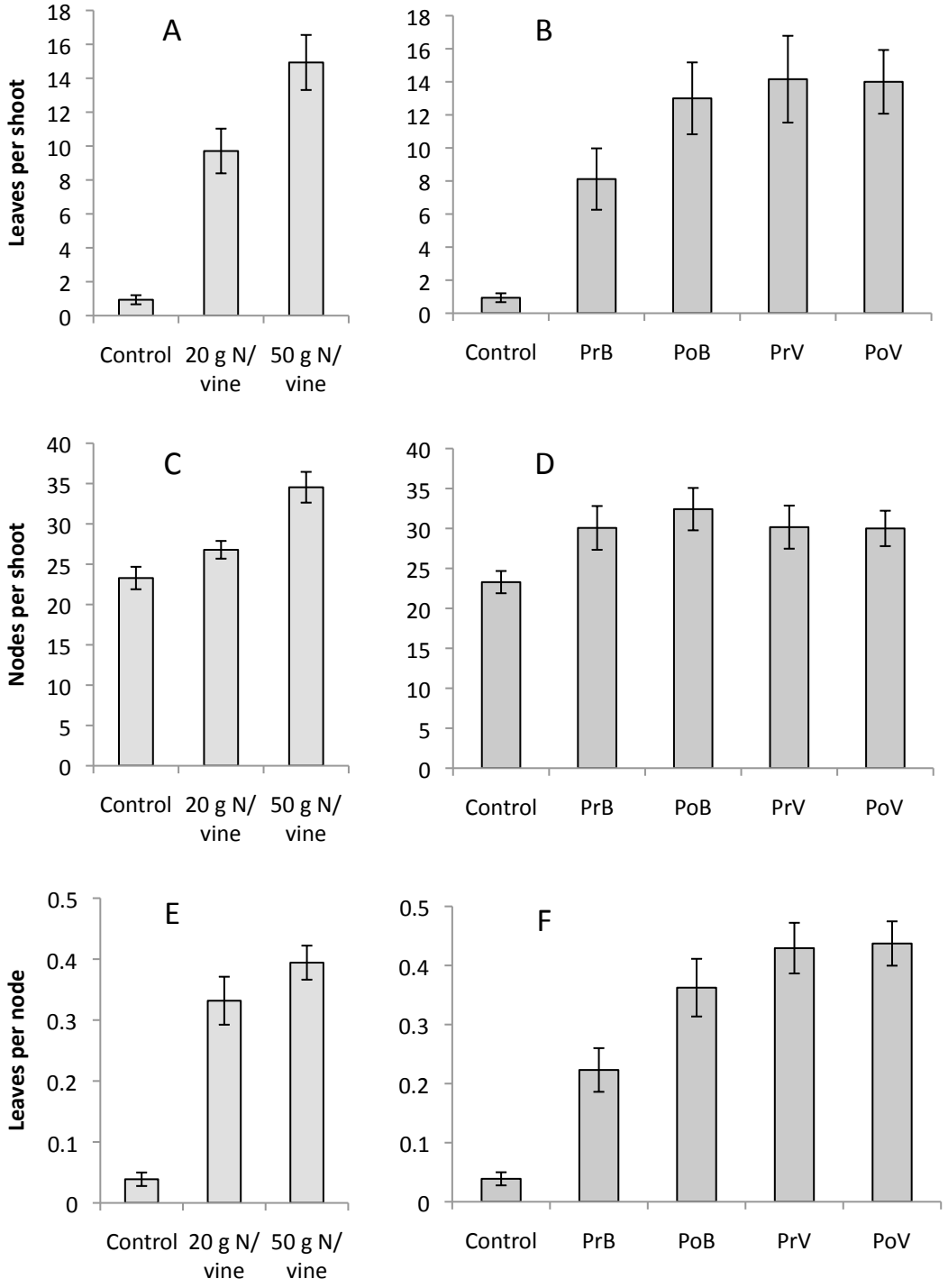

Figure 4.2-5 Impact of nitrogen application timing and rates in the second year of fertilisation (2007-08) on late season leaf counts. Timings were pre-bloom (PrB), post bloom (PoB), pre-veraison (PrV) and post veraison PoV). Rates were 20 g N/vine and 50 g N/vine. Controls were untreated. A, B – Leaves retained per shoot; C, D – Nodes per shoot; and E, F – Leaves per node. Error bars represent standard error of the mean.

Chlorophyll assessments were made on leaves on the main shoot at predetermined node positions, and leaf abscission from those nodes was recorded. These indicate that the pre-bloom additions had begun losing more main shoot leaves than other application timing treatments in early March (Figure 4.2-6). Prior to this date all treatments and the control had close to full leaf counts at the monitor nodes.

Post veraison applications were not significantly different to any application timing, until later in March, when it too remained higher than both the control and the pre-bloom application. This difference widened as the season progressed.

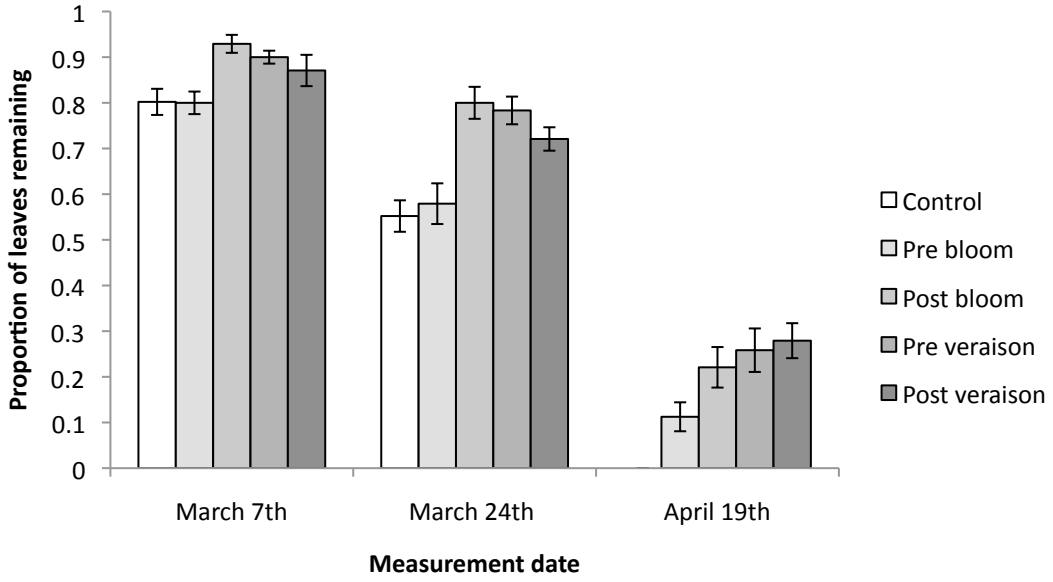

Figure 4.2-6 Leaf loss observed during chlorophyll assessment at three different dates, observed as a proportion of leaves remaining, in response to timing of nitrogen fertiliser application (no significant differences prior to March 7th). Error bars represent standard error of the mean.

The lack of significant difference from increasing nitrogen rates from 20 g N/vine to 50 g N/vine on leaf retention per node is clear at all chlorophyll concentration assessment dates from March 7th onwards (Figure 4.2-7). These counts are of main shoot leaves only, and there are no significant differences in leaf counts as a result of increasing the nitrogen rate from 20 g N/vine to 50 g N/vine.

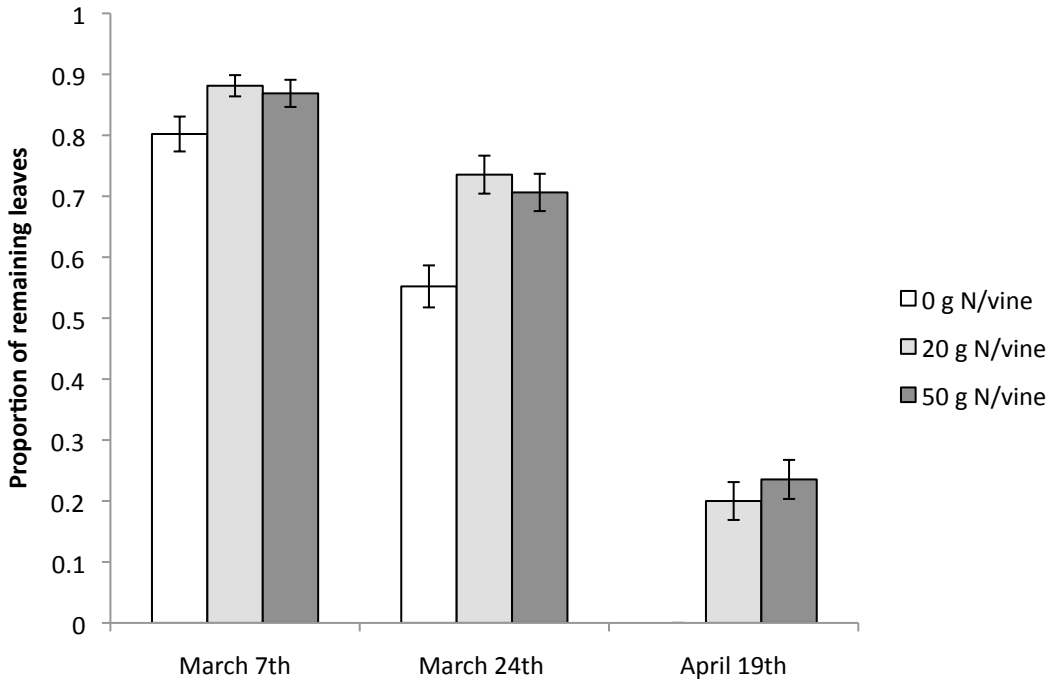

Figure 4.2-7 Leaf loss observed during chlorophyll assessment at three different dates in the 2007-08 season, observed as a proportion of leaves remaining, in response to different rates of nitrogen fertiliser addition. Error bars represent standard error of the mean.

The difference in lateral growth was clear from counts of the number of lateral shoots per main shoot, and nodes per shoot. The number of lateral shoots did not change significantly as a result of nitrogen application, however there was an increase in the number of nodes per lateral shoot following addition of 50 g N/vine (Table 4.2-5).

*Table 4.2-5 Impact of nitrogen addition on the number of lateral branches and the node count per lateral branch in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	*Lateral branches per cane*	*Nodes per lateral branch*
Control	13.0	2.1a
20	13.8	2.1a
50	14.9	2.5b
Sig	n.s.	***

Leaf chlorophyll concentration estimates

Vines that had received the highest rates of nitrogen addition had a higher CCI values meter in the year following the nitrogen by irrigation trial at leaf positions 4 and 6 than other rates, although there was no significant difference at node 9 (Table 4.2-6).

*Table 4.2-6 Leaf chlorophyll concentration estimation (CCI units) in the year following the nitrogen by irrigation trial, in response to application rate. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	Leaf 4 CCI	Leaf 6 CCI	Leaf 9 CCI
0	18.9a	15.9a	12.8
17	18.6a	16.1a	13.4
51	21.3b	18.2b	14.9
Sig	**	**	n.s.

Leaf chlorophyll levels in the first year of the nitrogen timing by rate trial (2006-07) were greater at veraison in vines that received nitrogen pre-bloom, post bloom and pre-veraison than the control vines and those that received nitrogen post veraison (Table 4.2-7). The highest rates were in vines that received nitrogen post bloom.

*Table 4.2-7 Leaf CCI averages at veraison in the 2006-07 season in response to nitrogen fertiliser timing. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N timing	CCI
Control	10.7a
PrB	17.4b
PoB	19.7c
PrV	17.3b
PoV	12.7a
Sig	***

*Table 4.2-8 Leaf chlorophyll estimations at veraison in the 2006-07 season in response to nitrogen fertiliser application rate. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	CCI
0	10.7a
20	16.1b
35	16.4b
50	17.9c
Sig	***

Analysis of the chlorophyll content estimates from 2007-08 contained a number of interactions, particularly between analysis date and fertilisation date ($P<0.001$), and analysis date and rate ($P=0.034$). In December and January in the 2007-08 season, there were no significant differences in CCI values between any treatments that received nitrogen, although all were greater than the control vines (Figure 4.2-8). Chlorophyll estimations in February and March of vines that received nitrogen post bloom were significantly higher than all other treatment timings, with the exception of the final assessment when only vines that received pre veraison nitrogen matched it.

Figure 4.2-8 also indicates a continual decrease in overall leaf chlorophyll levels as the season progressed.

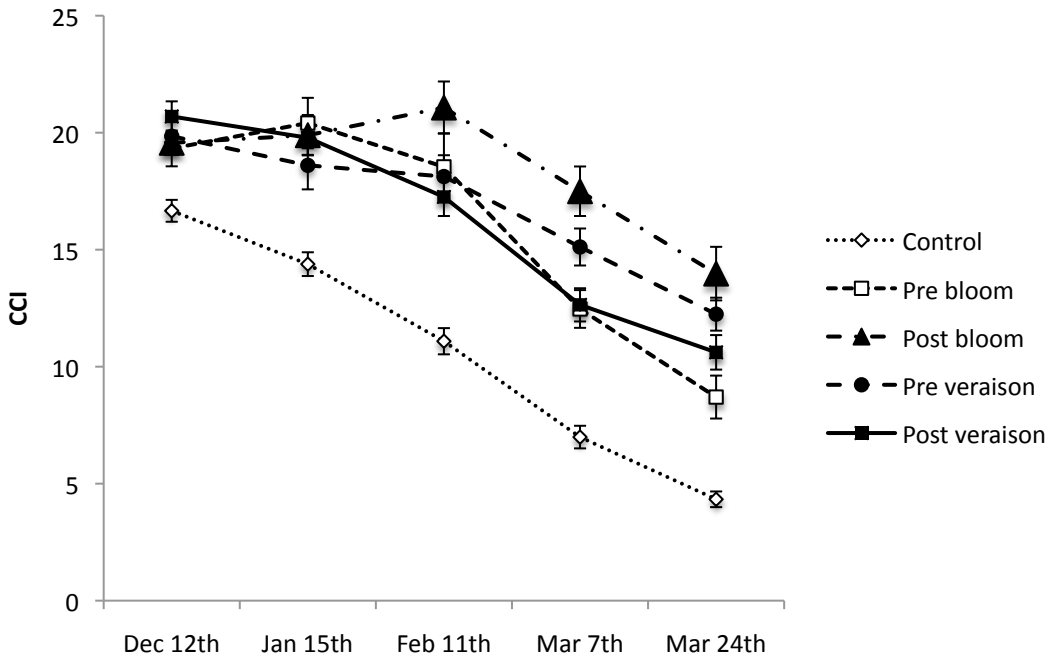

Figure 4.2-8 Leaf chlorophyll concentration estimates as CCI units throughout the 2007-08 season, in response to nitrogen application timing. Error bars represent standard error of the mean.

In December 2007, leaves lower on the shoot had a higher CCI value than those higher up (Figure 4.2-9). The CCI values of leaves at all heights were much closer in January and February. By March the 7th 2008 chlorophyll concentration increased with leaf height, and this disparity was even larger on March the 24th 2008. By these dates, all leaf heights were significantly different in CCI to the others.

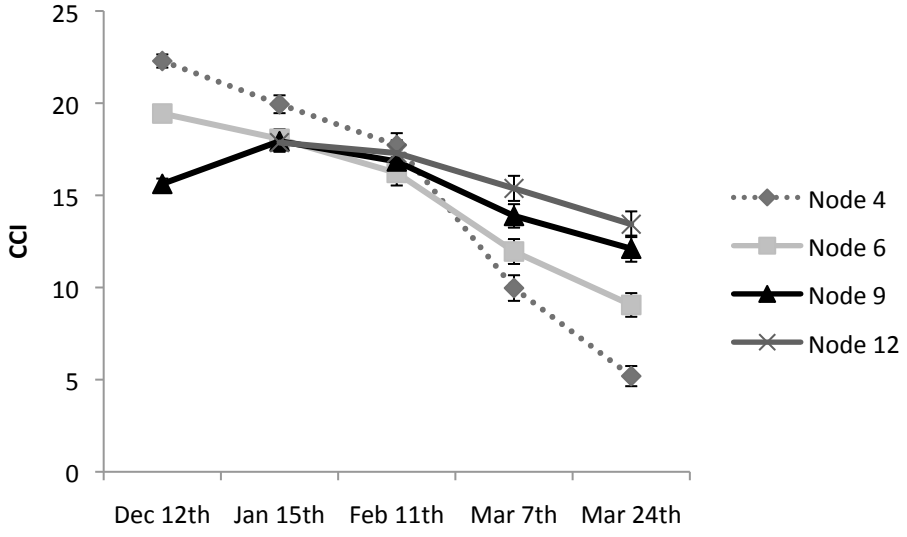

Figure 4.2-9 Leaf chlorophyll at different node positions through the 2007-08 growing season, averaged across all nitrogen treatments. Error bars represent standard error of the mean.

More applied nitrogen led to an increase in the CCI values at every date it was assessed (Figure 4.2-10). CCI decreased significantly in every measurement in the control vines (leading to the interaction effect). CCI did not change significantly until February 11th in

vine receiving 20 g N/vine, and March 7th in vines receiving 50 g N/vine.

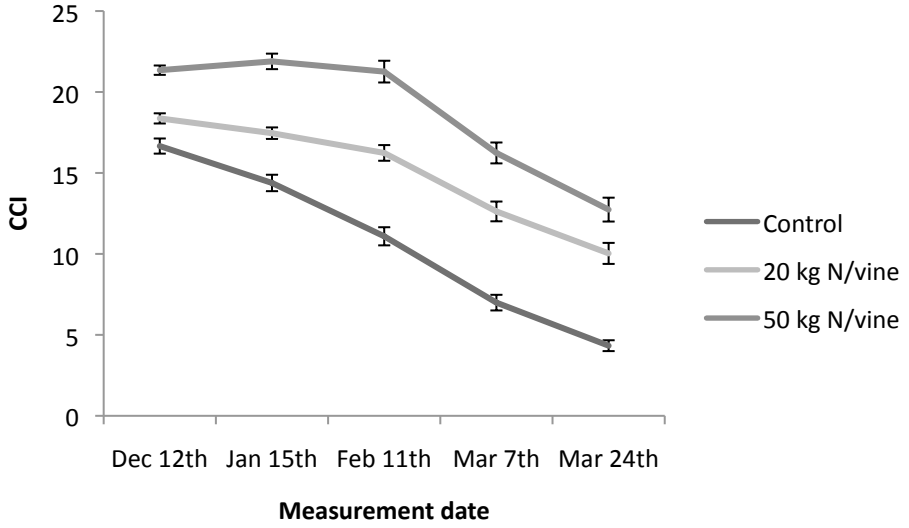

Figure 4.2-10 Leaf chlorophyll through the 2007-08 season as a result of different rates of nitrogen application. Error bars represent standard error of the mean.

Leaf light penetration

Leaf chlorophyll index was correlated to the transmission of photosynthetically active radiation (PAR) through the leaf (Figure 4.2-11). Leaves with a higher CCI value transmitted less light through the leaf. Leaves that were yellow in colour with no visible chlorophyll transmitted around 50% of the light that they were exposed to.

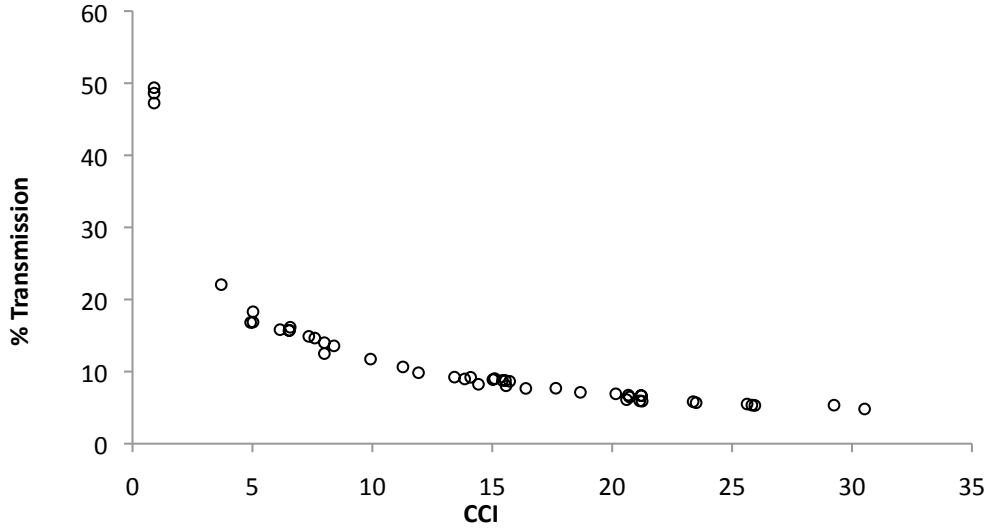

Figure 4.2-11 Percentage of photosynthetically active radiation (PAR) transmitted through leaves with varying chlorophyll content index (CCI). Leaves were sourced from the nitrogen timing by rate trial in 2007-08

Leaf area

Leaf area for individual leaf sizes was assessed in the 2007-08 season. Vines receiving nitrogen additions of 50 g N/vine had leaf areas of 202 cm^2, higher than all other

treatments. 20 g N/vine produced leaves that were not significantly different to the control vines, at 179 cm^2 and 175 cm^2 respectively.

Leaf nitrogen levels

The timing of nitrogen in the preceding season influenced leaf nitrogen concentrations at bloom in the second year of the nitrogen timing by rate trial, with applications made later in the season increasing lamina nitrogen concentration to a greater degree. Post veraison applications led to the highest nitrogen concentrations, while pre-bloom applications were not different to the levels of the control vines (Table 4.2-10). Veraison sampling of leaves indicated that the highest leaf nitrogen concentrations were on vines that received post bloom or pre-veraison nitrogen (Table 4.2-10). All leaves that had received nitrogen, regardless of timing or rate, had higher nitrogen concentrations at veraison than the control.

Higher rates of nitrogen led to more leaf nitrogen in the leaf at both sampling dates, although control vines and vines that had received 20 g N per vine did not differ significantly when measured at bloom (Table 4.2-9).

*Table 4.2-9 Impact of nitrogen fertiliser rate on the of leaf lamina nitrogen percentage at veraison in 2006-07, and at bloom and veraison in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate	*2006-07 Veraison lamina nitrogen %*	*2007-08 Bloom lamina nitrogen %*	*2007-08 Veraison lamina nitrogen %*
0	1.57a	3.15a	1.64a
20	1.87b	3.22a	1.98b
50	2.01c	3.46b	2.18c
Sig	***	***	***

*Table 4.2-10 Impact of nitrogen application timing on the of leaf lamina nitrogen percentage at veraison in 2006-07, and at bloom and veraison in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	*2006-07 Veraison lamina nitrogen %*	*2007-08 Bloom lamina nitrogen %*	*2007-08 Veraison lamina nitrogen %*
Control	1.57a	3.15a	1.64a
Pre-bloom	1.90b	3.15a	2.02b
Post bloom	2.02b	3.31b	2.17c
Pre veraison	1.89b	3.38b	2.14c
Post veraison	-	3.53c	1.99b
Sig	***	***	**

There were positive correlations between leaf nitrogen and CCI values (Table 4.2-11). Bloom nitrogen levels were correlated to December chlorophyll concentration estimations ($R^2 = 0.54$). Veraison nitrogen levels were most strongly correlated to February chlorophyll concentration estimations ($R^2 = 0.68$; Figure 4.2-12). Correlations between leaf nitrogen and leaf chlorophyll measured at other dates decreased as the interval between the two samples increased (Table 4.2-11).

*Table 4.2-11 R^2 values for regression analysis between leaf lamina nitrogen concentration, and vine average chlorophyll concentration estimated by CCM200 chlorophyll meter. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Lamina sampling date	*December*	*January*	*February*	*March*	*April*
Bloom	0.54***	0.36***	0.24***	0.30***	0.20***
Veraison	0.52***	0.55***	0.68***	0.55***	0.38***

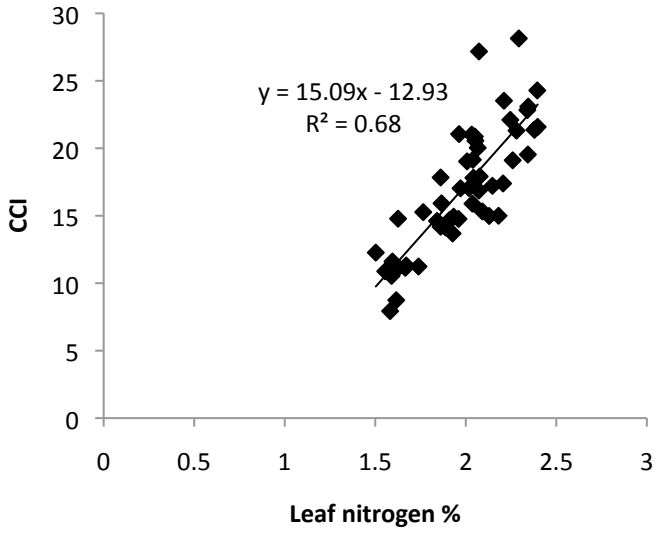

Figure 4.2-12 Leaf chlorophyll concentration estimation by CCM200 meter in February 2008 in CCI units against leaf nitrogen percentage from veraison leaf lamina samples

Leaf anthocyanin

In the 2006-07 season, leaves were seen to develop a red colour towards the end of the season in response to nitrogen addition, indicating a build up of anthocyanin. The leaves were observed to initially develop red colouration along the leaf veins, and then out across the leaf lamina. Microscopic examination indicated that there were deposits of red pigment concentrated in the palisade mesophyll cells in leaves with red lamina.

Leaf red colouration was limited to vines that received pre-bloom applications of nitrogen, leading to a significant interaction effect between nitrogen application timing and rate ($P<0.001$) with no significant difference between any other nitrogen treatment. Table 4.2-12 shows the increase in leaf redness with increasing nitrogen rate, for the pre-bloom application only.

*Table 4.2-12 Impact of nitrogen rate on leaf counts of leaves with visible anthocyanin within the pre-bloom nitrogen addition treatment in 2006-07. No other timing led to a result significantly different to the control. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	***Leaves with over 50% red veins***
0	0a
20	2.7b
35	2.8b
50	6.8c
Sig	***

In 2007-08 there was again a significant interaction response (P=0.001), with pre-bloom applications of 20 g N/vine having less red leaves than other dates at that rate, while no timings of 50 g N/vine were different to each other. There was a significant increase in response to the rate of nitrogen application. (Table 4.2-13) In this season a leaf colour scorecard was used instead of a leaf counting system.

*Table 4.2-13 Leaf redness judged by scorecard in 2007-08 in response to nitrogen application rate, at the 17th of April. (Redness score: 1 = No visible redness; 5 = Lamina surface red). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	***Redness score***
0	1.0a
20	2.3b
50	3.8c
Sig	***

Shoot growth

Bud burst

Bud burst was increased by nitrogen addition in all years of the trial (Table 4.2-14, Table 4.2-15, Table 4.2-16). Vines that received nitrogen in the nitrogen by irrigation trial in 2005-06 trial showed an increase in bud burst in the 2006-07 season as a result of nitrogen addition, however there was no increases from increasing the nitrogen level from 17 to 51 g N/vine (Table 4.2-14). There was no impact on bud burst from different levels of irrigation between veraison and the end of the preceding season.

Bud burst was increased by nitrogen addition in 2006-07, although there was no rate or timing effect (Table 4.2-15). Nitrogen addition the preceding season led to a reduction in the number of nodes per arm, and as a result there was no overall difference in shoot number in 2007-08.

There was a reduction in nodes per arm in 2008-09 in vines treated with 50 g N/vine the preceding season, however there was also a large increase in percent bud burst (Table 4.2-16). As a result, there are more shoots per arm on vines receiving either nitrogen rate than the control vines.

*Table 4.2-14 Bud burst in 2006-07 in response to nitrogen fertiliser addition in veraison the preceding season. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Nodes per arm	Blank nodes	Buds burst	Shoots per arm	Bud burst (%)
0	10.4	4.4c	6.1a	6.1a	58a
17	10.5	3.6b	6.9b	7.0b	66b
51	9.7	3.1a	6.6b	6.8b	68b
Sig	n.s.	**	**	**	***

*Table 4.2-15 Bud burst in 2007-08 in response to nitrogen fertiliser addition in the preceding season in the nitrogen timing by rate trial. No significant differences were observed between different rates of nitrogen or different application timing. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Nodes per arm	Blank nodes	Shoots per arm	Bud burst (%)
0	10.2b	4.3b	5.9	58a
20	9.7a	3.8a	5.9	61b
50	9.6a	3.5a	6.1	64b
Sig	*	**	n.s.	*

*Table 4.2-16 Bud burst in 2008-09 in response to nitrogen fertiliser addition in the preceding season. No significant differences were observed between different application timings. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Nodes per arm	Blank nodes	Buds burst	Shoots per arm	Bud burst (%)
0	9.1b	4.5c	4.6a	4.7a	51a
20	8.9b	3.6b	5.3b	5.6b	60b
50	8.2a	2.8a	5.4b	5.6b	66c
Sig	**	***	***	***	**

Early season shoot growth

Shoot length in the 2006-07 season increased as a result of nitrogen added at veraison in 2005-06 in the nitrogen and irrigation trial (Table 4.2-17) at both assessment dates. Average shoot E-L stage and leaf counts increased following nitrogen addition, although there was no significance between the two different rates of nitrogen application. The shoot extension continued to increases between the two different measurement dates, as well as the leaf counts (Table 4.2-18).

*Table 4.2-17 Shoot length and leaf counts in 2006, the year following fertilisation in the nitrogen and irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Assessment date	N rate (g N/vine)	E-L	Shoot length (mm)	Leaves per shoot
15th Oct	0	13.3a	177a	6.4a
	17	13.7ab	211b	6.7ab
	51	14.2b	234c	7.2b
	Sig	**	***	**
30th Nov	0	16.6a	473a	11.2a
	17	17.1b	568b	12.4b
	51	17.3b	670c	12.9b
	Sig	*	***	***

*Table 4.2-18 Change in leaf counts and shoot length between assessment dates in 2006, the year following fertilisation in the nitrogen and irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	Δ Shoot length	Δ Total leaves
0	296a	4.9a
17	357a	5.7b
51	435b	5.7b
Sig	***	*

Supplemental irrigation led to a reduction in shoot length (Table 4.2-19), assessed on the 30th of November. Irrigation rates did not affect the E-L measurements or leaf counts.

*Table 4.2-19 Shoot length in response to changes in irrigation rate in 2006, the year following establishment, in the nitrogen and irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Irrigation	Early shoot length	30th Nov shoot length
Nil	204	589b
Normal	210	605b
High	191	546a
Sig	n.s.	*

The average E-L of monitor shoots in 2007-08 was increased by nitrogen fertilisation in 2006-07 in the nitrogen rate by timing trial. The distribution of growth stages was altered by nitrogen application, as can be seen in Figure 4.2-13. Unfertilised vines had more shoots that were less developed, while addition of nitrogen increased the number of shoots that were at the maximum E-L stage measured at this date. There was also an impact from increasing nitrogen rate on the number of shoots that were maturing at the maximum rate, as is indicated in Figure 4.2-14.

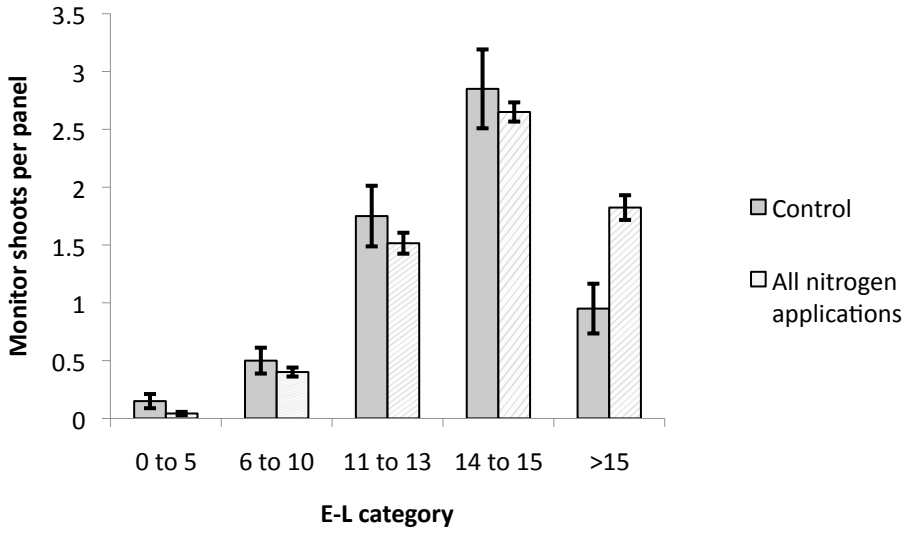

Figure 4.2-13 Average shoot count per vine arm falling into different E-L categories of untreated control vines and the average of all vines treated with nitrogen in the 2006-07 season, assessed on the 6th of November, 2007. Error bars represent standard error of the mean.

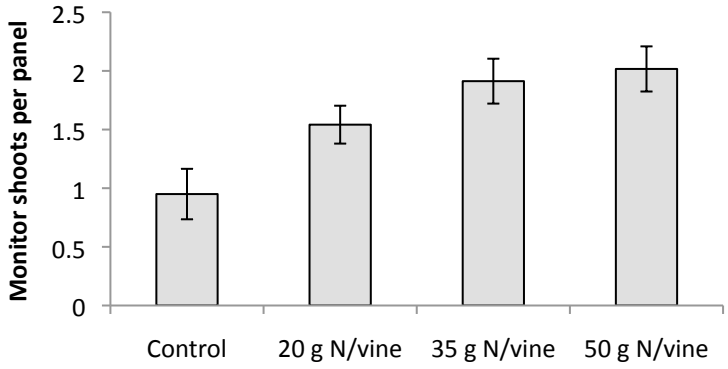

Figure 4.2-14 Number of monitor shoots per panel at E-L stage 15 or over on the 6th of November 2007 as a result of increasing nitrogen rate applied in 2006-07. Error bars represent standard error of the mean.

Shoot tip activity

Pre-bloom applications of nitrogen in 2006 led to an increased level of shoot tip activity in the middle of January 2007 (Figure 4.2-15), averaged across all shoots measured.

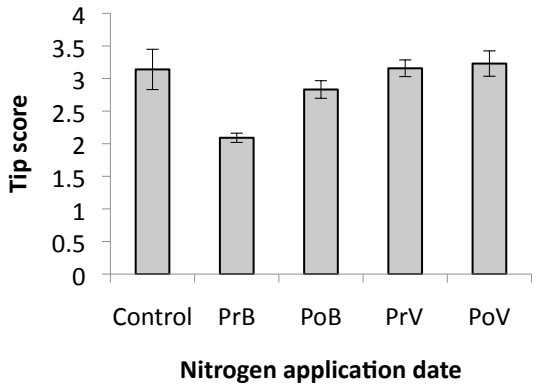

Figure 4.2-15 Impact of nitrogen application timing on shoot tip activity on 8th of January 2007 (lower score means more active growing tip). Error bars represent standard error of the mean.

Shoot maturation progression

Periderm browning was decreased by high rates of nitrogen applied at veraison in the 2005-06 season (Table 4.2-20). There was no change in periderm browning as a result of different rates of irrigation.

*Table 4.2-20 Shoots per vine with incomplete periderm browning on the 12th May 2006 following nitrogen application at different rates at veraison. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate	*Shoots*
0	0
17	0.8
51	2.3
Sig	**

There were no significant differences in the development of shoot maturation at veraison in the nitrogen timing by rate trial on the monitor shoots in the 2006-07 season in response to either rate (data not presented) or timing (Table 4.2-21). There was a non-significant trend towards reduced periderm browning of pre-bloom and post bloom nitrogen applications, compared to pre-veraison or control vines (P=0.096).

In the 2007-08 season, pre-veraison application led to a significantly greater number of brown internodes per monitor shoot than all other timings except the pre-bloom application (Table 4.2-21). Considering the pre-veraison application would not have had long to influence lignification, the pre-veraison application treatments represent the longest time between nitrogen application and periderm assessment.

*Table 4.2-21 Brown internodes as a result of nitrogen application timing in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	*Brown internodes 2007*	*Brown internodes 2008*
Control	5.7	6.5a
Pre-bloom	4.3	7.5ab
Post bloom	4.2	6.4a
Pre-veraison	5.4	9.5b
Post veraison	-	6.0a
Sig	n.s.	*

Pruning weights

Nitrogen applied at veraison in 2005-06 season did not lead to any change in vine pruning weights in that season, however there was a response in the following year (Table 4.2-22).

*Table 4.2-22 Pruning weight in 2007 following nitrogen addition in the 2005-06 season as part of the nitrogen by irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	*Pruning weight per vine (kg)*	*Mean cane weight (g)*
0	0.75a	28a
17	0.92b	33b
51	1.35c	46c
Sig	***	***

Pruning weights in the winter of 2007 were increased by pre-bloom nitrogen application of nitrogen, (Table 4.2-23), but not altered by other application timings. There were no significant differences as a result of increasing the rate of nitrogen.

*Table 4.2-23 Pruning weight response to timing of nitrogen application in the first year of the nitrogen timing by rate trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	*Pruning weight per vine (kg)*	*Mean cane weight (g)*
Control	0.83a	31a
PrB	1.22b	45b
PoB	0.89a	33a
PrV	0.90a	32a
PoV	0.81a	31a
Sig	***	***

In the second year of the nitrogen rate by timing trial, application timing did not affect pruning weight or mean cane weight, although all applications did increase the pruning weight above the control vines (Table 4.2-24). There was a response to increased nitrogen rates in this season, which led to increases in pruning weight and mean cane weight (Table 4.2-25).

*Table 4.2-24 Pruning weight in 2008 in response to timing of nitrogen application in the nitrogen timing by rate trial following two years of nitrogen addition. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	Pruning weight per vine (kg)	Mean cane weight (g)
Control	1.18a	45a
PrB	1.64b	58b
PoB	1.79b	63b
PrV	1.60b	55b
PoV	1.80b	63b
Sig	***	***

*Table 4.2-25 Pruning weight in 2008 in response to the rate of nitrogen application in the nitrogen timing by rate trial following two years of nitrogen addition. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	Pruning weight per vine (kg)	Mean cane weight (g)
0	1.19a	45a
20	1.54b	53b
50	1.88c	66c
Sig	***	***

Canopy density measurements

Point quadrat

Both nitrogen and irrigation rates applied at veraison in 2005-06 had an impact on vine canopy gaps and fruit exposure, although nitrogen rate did not impact canopy density, as estimated by the vigour scorecard (Table 4.2-2, Table 4.2-3).

Point quadrat analysis in 2007-08 showed that there were more internal, external and total leaves on vines that received increased rates of nitrogen fertiliser. Nitrogen addition also decreased the number of yellow leaves in the canopy (Table 4.2-26).

*Table 4.2-26 Changes in point quadrat parameters as a result of different nitrogen fertiliser rates in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	Occlusion layer	% Internal leaves	Yellow leaves	Leaf Layer Number	Leaf exposure layer	Percent gaps	Internal clusters	Cluster exposure layer
0	1.9a	13.7a	26.7a	1.8a	0.25a	7.0a	40	0.06a
20	2.2b	21.9b	12.1b	2.1b	0.48b	6.4a	39	0.06a
50	2.7c	28.3c	13.6b	2.4c	0.76c	2.8b	55	0.13b
Sig	***	***	***	***	***	*	n.s.	**

There were no significant differences for any of the point quadrat parameters measured as a result of changing the nitrogen timing apart from the assessment of yellow leaves. Pre-bloom application treatment vines had more yellow leaves than vines that received nitrogen at any other timing (Figure 4.2-16), and was not significantly different to the control vines.

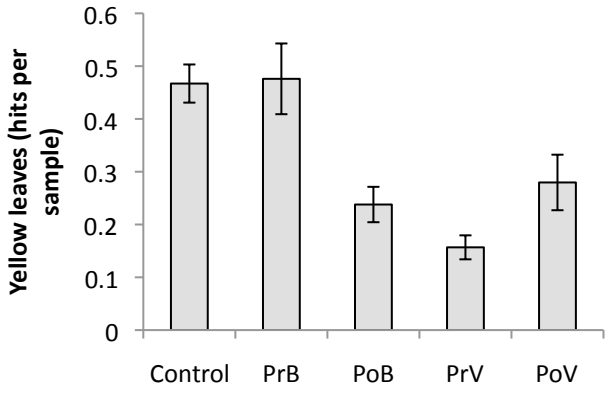

Figure 4.2-16 Average yellow leaves per point quadrat insertion point in 2007-08 season in response to date of application of nitrogen. Error bars represent standard error of the mean.

Canopy light penetration

Canopy light penetration in the 2007-08 season showed that there was a decrease in light in the fruiting zone as a result of nitrogen application, which increased with rate (Table 4.2-27). There was no significant difference between the different application timings.

*Table 4.2-27 Changes in interior photosynthetically active radiation light in response to nitrogen rate, assessed by LiCor light meter in late February 2008. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N rate (g N/vine)	*Percent light inside canopy*
0	15.1a
20	11.0b
50	7.9c
Sig	*

4.2d Discussion

Nitrogen and Irrigation

Whilst moisture stress and nitrogen deficits can both limit vine growth (Keller, 2004), and induce senescence processes (Gan and Amasino, 1997, Lim et al., 2007), in this study it was found that supplemental irrigation had much less impact on late season leaf health than addition of nitrogen (Table 4.2-2, Table 4.2-3, Table 4.2-4). Vine leaf retention and canopy vigour increased with nitrogen fertilisation. However, while decreasing soil moisture through withholding irrigation did induce a reduction in canopy growth, additional irrigation did not improve the canopy health. It can be concluded from these findings that a deficit of nitrogen, and not of moisture, was limiting late season leaf health in this block. As a result of these observations, subsequent trials examined the impact of supplemental nitrogen on the vine growth.

No significant interactions were noted between irrigation rate and nitrogen availability, although such interactions can occur (Keller, 2004), with increasing moisture potentially increasing nutrient availability through a greater root zone. Alternatively, irrigation that exceeds field capacity for the root zone will lead to leaching of nitrogen, reducing the total pool of nitrogen available to the plant (Schaller, 1991, Löhnertz, 1991). The lack of interaction may be due to the short duration of this trial. Also, neither soil water use or

water potential were monitored during the trial, therefore it is not possible to determine whether the increased water rates led to stress from water logging, increased leaching or other negative impacts from excessive soil moisture.

The discussion that follows is divided into the results from within the initial year of application, and the results from years subsequent to this.

Nitrogen Timing by Rate trial

Leaf Chlorophyll and Leaf Retention

The estimates of leaf chlorophyll concentration were significantly correlated to leaf nitrogen concentration (Table 4.2-11). The strong link between leaf chlorophyll and nitrogen concentration has been noted in other studies, and chlorophyll has even been suggested as an indicator for determining leaf nitrogen in grapevines (Spring and Jelmini, 2002, Candolfi-Vasconcelos et al., 1997). Poni et al (1994) studied links between nitrogen and chlorophyll concentration, and photosynthesis rates, and found that nitrogen content on a dry weight basis was significantly correlated to photosynthesis rates in mature leaves, but chlorophyll concentration did not correlate to photosynthesis rates in leaves of any maturity. The leaves used in the monitoring were mature leaves therefore it is possible that the increases in nitrogen following the addition of fertilisers may have been associated with an increase in leaf photosynthesis rate, although this was not measured.

Leaf retention was strongly influenced by the timing of nitrogen addition in both 2006-07 and 2007-08. Increases in late season leaf retention were not significantly different between post bloom, pre-veraison and post veraison nitrogen additions in both seasons, while pre-bloom nitrogen additions were significantly less (Figure 4.2-1, Figure 4.2-4, Figure 4.2-5). Increases in leaf chlorophyll from nitrogen additions made bloom, post bloom and pre-veraison were apparent by the time of veraison in 2006-07 (Table 4.2-7). Post bloom applications lead to the highest chlorophyll concentration at veraison in 2006-07 and in February and early March in 2007-08. The impact of the timing of nitrogen application on vine shoot growth has been investigated by other researchers (Bettiga and West, 1991, Christensen et al., 1994, Conradie, 2001, Holzapfel and Treeby, 2007, Peacock et al., 1991), and increases in leaf chlorophyll (Candolfi-Vasconcelos et al., 1997, Keller, 2004, Spring, 2002, Spring and Jelmini, 2002) and photosynthesis (Vasconcelos et al., 2005) as a result of nitrogen application have also been reported. However few trials link nitrogen timing with different leaf health responses. This information may be a benefit in vineyards where late season leaf health declines prior to harvest, as can occur in cool climate viticulture.

Plants can respond quickly to increased soil nitrogen. Nitrate, the preferred form of uptake for the grapevine (Roubelakis-Angelakis, 1991, Wermelinger, 1991, Gu et al., 1996), was provided by the EasyN fertiliser additions in 2006-07 and 2007-08. The vines that received nitrogen at the pre-veraison timing in 2006-07 demonstrate the speed of nitrogen uptake, with increases in leaf chlorophyll observed twenty days after application (Table 4.2-7). Increasing nitrogen availability will stimulate the production of cytokinins in the roots, which will act as a signal to the rest of the plant that there is a nitrogen source present (Sakakibara, 2006). Addition of cytokinin to plants that would otherwise be senescing has been shown to inhibit (Gan and Amasino, 1995) and even reverse senescence processes (Zavaleta-Mancera et al., 1999a, Zavaleta-Mancera et al., 1999b). Slowing down senescence will decrease the breakdown of chlorophyll (Hörtensteiner, 2006) and delay abscission. The impact of nitrogen on leaf senescence has been demonstrated previously on grape vines (Keller et al., 1998), and was evident in this trial from delayed abscission.

Early in the 2007-08 season, CCI values where higher for lower leaf positions, however as the season progressed there was a continuing decline in the CCI values from lower, older leaves, while the CCI values increased on leaves at node 9 on the shoot up until January. By March, leaf CCI values increased with increasing leaf node number (Figure 4.2-9). Increasing nitrogen application rate did not alter this pattern, leading to an increase in CCI values at all measurement dates (Figure 4.2-10). The average CCI value of all measured leaves on vines that received 50 g N/vine did not start to decrease until the March assessments, whereas the same attribute decreased in both the control and the 20 g N/vine treatments from December onwards. From this it can be concluded that the 50 g N/vine treatments led to a delay in senescence processes. Nitrogen is remobilised during senescence (Hörtensteiner and Feller, 2002), and export has been observed commencing in grape vine leaves of around 50 days age (Poni et al., 1994). Nitrogen export is such a fundamental characteristic of senescence that Masclaux et al. (2000) suggest it can be used as a definition for the onset of senescence. The reduction in nitrogen is then followed by a reduction in leaf chlorophyll, which has been observed to decrease in leaves over 80-90 days old (Poni et al., 1994, Bertamini and Nedunchezhian, 2002). The reduction in chlorophyll is delayed because it reduces in response to the lack of nitrogen, not concurrently (Hörtensteiner, 2006). This may reduce the correlation between nitrogen and chlorophyll, however the relationship between the two is reasonably robust.

Nitrogen levels in leaf lamina at bloom were highest for post veraison nitrogen timings, with levels progressively decreasing with earlier timings. Pre-bloom applications had no impact on leaf lamina nitrogen concentration sampled at bloom. Peacock et al (Peacock et al., 1991) found that while additions of nitrogen prior to bloom had some impact on leaf nitrogen concentration at bloom, post harvest additions were the most effective, and as in this trial levels decreased with earlier timings. By veraison however, the nitrogen additions within the current season were influencing lamina nitrogen concentration to a greater degree than additions made in the preceding season, with the post bloom and pre-veraison applications having the highest nitrogen concentration within the lamina. Nonetheless, post veraison applications still gave rise to significantly higher laminar nitrogen concentration than that of control treatment vines, even though 12 months had passed between the last nitrogen application on these vines (Table 4.2-10). Adequate levels of nitrogen in lamina at bloom are given as 3.9-5.0% and are critical below 2.9% (Robinson, 1992). Therefore, the vines in this trial are all below the adequate level, but above critical. Veraison adequate values are 2.2-4.0, and are critical below 1.6% (Robinson, 1992). Leaves from all treatments, including the high rates of nitrogen addition, are still below the level defined as adequate.

The ability of leaves to photosynthesise may be reduced when nitrogen is in deficit. Adding nitrogen had the impact of increasing leaf area (through both larger leaves, and more leaves), as well as increasing the chlorophyll concentration of those leaves. This can lead to improved photosynthetic capacity (Candolfi-Vasconcelos et al., 1994). In cooler years, when reduced temperatures and light availability may limit carbohydrate production and fruit ripening, an increased ability to photosynthesise may confer a production advantage.

Shoot growth

Nitrogen fertilisation increased the percentage of buds that burst in every season following all nitrogen applications (Table 4.2-14, Table 4.2-15, Table 4.2-16). A similar effect was implied in the study of Löhnertz (1991), where bunches per vine were increased as a result of nitrogen addition, without increasing bunches per shoot, and

with no significant differences in bud number per vine or per metre of cordon. Moderate rates of nitrogen also increased the bud burst in the study of Kliewer *et al.* (1991), by correcting a 'reduced capacity' in the unfertilised vines. Lombard et al. (2006) identified a peak in cytokinin prior to budburst, with higher levels of cytokinin being linked to more advanced bud burst. They also found signs that the cytokinin was not originating from the roots in the previous season, and suggest that it may be stored in the woody tissue over winter. Cytokinins will rise when nitrogen availability increases (Forde, 2002). Further research into the impact of nitrogen availability on stored cytokinins is required to increase the understanding of the links between nitrogen and budburst.

The increase in bud burst did not always lead to an increase in shoots per vine. Although this was observed in the season following the irrigation by nitrogen trial (Table 4.2-14), and in the season following the second application of nitrogen in the rate by application timing trial (Table 4.2-15), it was not evident in the year following the first season of the timing by rate trial (Table 4.2-15). Nitrogen application will increase shoot extension and internode length (Winkler, 1970), which led to reduced nodes per arm left after pruning in every season in response to nitrogen addition, which reduced the potential shoot numbers. The pruning in this block was designed to fill the fruiting wire, and therefore canes with a reduced internode length will have more nodes laid down. It was only in the 2008-09 season that there was a difference in bud burst percentage from increasing the nitrogen rate from 20 g N/vine to 50 g N/vine (Table 4.2-16). However, the higher nitrogen treatment in this season also resulted in a decrease in the nodes per arm compared to both the control and the 20 g N/vine, leading to no difference in shoots per vine.

Early season shoot growth rate averages were higher on vines that had received nitrogen the previous season. Although maximum shoot node numbers did not increase as a result of nitrogen addition, there were more shoots reaching the maximum level of development (Figure 4.2-14). The main factor impacting the appearance of nodes on a shoot is temperature (Moncur et al., 1989) however decreased levels of stored carbohydrates may lead to a reduction in appearance of nodes (Greer and Weston, 2010). Nitrogen impacts on shoot length have been reported by a number of other authors (Bell and Robson, 1999, Ewart and Kliewer, 1977, Shawky et al., 2004), however little has been reported on the impact of nitrogen on early season rates of node appearance.

Plant nitrogen availability prior to fruit set was a major factor in determining vine shoot growth. In 2006-07 this was observed as increased shoot growth in vines receiving pre-bloom nitrogen treatments. In 2007-08, timing of application had no impact on vine growth, however leaf nitrogen concentrations at bloom indicated that there was more nitrogen within the vines that had received nitrogen post bloom, pre-veraison or post veraison in the previous season (Table 4.2-10). Nitrogen can be stored in trunks and roots, principally in the form of arginine (Wermelinger, 1991) and these stores are used in early season growth (Araujo and Williams, 1988, Wermelinger, 1991, Conradie, 1991). The amount of stored nitrogen available to early season growth can be increased by higher nitrogen availability during the preceding season (Löhnertz, 1991), however fruit can compete with the storage organs for nitrogen. Depletion of stored nitrogen ceases around bloom (Zapata et al., 2004), and storage will increase as the season progresses, to a maximum rate in the later stages of the season (Wermelinger, 1991). Therefore, the later season applications of nitrogen coincide with periods of increased nitrogen storage when fruit are less able to compete with the wood as a nitrogen sink. Although stored nitrogen was not measured in this trial, this is the probable cause of the increases in bloom lamina nitrogen levels. Pre-bloom applications did not increase bloom lamina nitrogen, however as demonstrated in the previous season, nitrogen applied at this stage

led to increased growth within the same season. In addition to retarding senescence, cytokinins produced in response to increased nitrogen availability can lead to increased shoot growth (Sakakibara, 2006, Zavaleta-Mancera et al., 1999b). Prior to bloom the vine shoot growth is in an active stage, and addition of nitrogen led to this growth continuing on for longer than other treatments. Growth had started to slow by the time of the post bloom application in 2006-07, as could be seen in the shoot tip activity survey (Figure 4.2-15) conducted one week following the post bloom addition.

Shoot maturation can be assessed by periderm browning (Keller, 2010), and can be an important factor in determining frost hardiness, particularly in cool climates. Periderm browning decreased with higher nitrogen addition in 2005-06, which is one of the effects of high nitrogen mentioned by Winkler (1970). The pre-veraison nitrogen applications had the highest number of brown internodes per shoot in 2007-08, however there were no rate effects in either year of the timing by rate trial. Water stress has also been linked to increased periderm browning (Matthews et al., 1987), where periderm browning was linked to the cessation of shoot growth. There was no impact on periderm browning from altering irrigation levels after veraison in 2005-06, however there were links between nitrogen and shoot growth in the nitrogen rate by timing trial and this may be linked to the development of brown periderm. Shoot maturation may be linked to increased cold hardiness (Wample and Wolf, 1996), however there is little information in the literature on periderm browning, and its importance to wine quality.

The mean cane weights in both nitrogen trials in 2006-07 indicate that all vines in this trial were in the range classified by Smart and Robinson (1991) as moderate vigour vines. In 2007-08, mean cane weights indicated that these vines were high vigour vines, although Kliewer and Dokoozlian (2005) suggest the optimal pruning weight values could be higher in cool climate Pinot Noir production. There is evidence of a seasonal difference, since control vines in 2007-08 had mean cane weights equal to the pre-bloom treatments in 2006-07, and were 50% greater than the control vines of 2006-07. Seasonal changes can increase nitrogen availability, but may also increase soil moisture availability and temperature, both of which can increase shoot growth (Keller, 2004, Keller, 2010).

Canopy density

Point quadrat assessment of canopy density increased with nitrogen rate (Table 4.2-26). There was a reduction in photosynthetically active radiation entering the fruiting zone (Table 4.2-27), due to increased leaf layer numbers, and potentially also as a result of increased leaf chlorophyll concentration (Figure 4.2-11). Light interception by fruit can have a major impact on wine quality (Smart, 1985). Increased interception of solar radiation will increase production of tannins (Smart, 1991, Cortell and Kennedy, 2006, Spayd et al., 2002), while increased berry temperature can alter acids (Bergqvist et al., 2001) and anthocyanins (Guidoni et al., 2008, Spayd et al., 2002) in the berry.

Leaf layer numbers did not vary between application timing treatments in 2007-08, although in the pre-bloom treatments a greater proportion of those leaves were yellow, indicating they were in the final stage of senescence. Leaf CCI value increases led to decreases in light transmission through the leaf, therefore the interior of canopies with more yellow leaves may have an increased light penetration even though leaf layer numbers may not vary.

Leaf Anthocyanin Production

An unexpected observation was the appearance late in the season of vines with leaves displaying an obvious increase in red colour of the veins. This was much more notable

on pre-bloom applications of nitrogen and was also much increased by higher rates of nitrogen (Table 4.2-12).

Dissection of the leaves revealed the pigment, presumed to be anthocyanin, was building up initially in the phloem, and would then spread through the leaf lamina, primarily in the palisade mesophyll cells.

Anthocyanin production is commonly observed in concert with leaf senescence, however the two are driven by genetically independent processes (Diaz et al., 2006). There is some debate over what factors are responsible for stimulating leaf anthocyanin production. Anthocyanin accumulation may be observed in leaves at a range of developmental stages. Proposed functions of anthocyanin accumulation include osmotic adjustment in response to drought or stress, acting as a protective antioxidant, or as a sunscreen to block UV and/or visible light (Close and Beadle, 2003, van den Berg and Perkins, 2007). It has been associated with delayed abscission, which may allow for an extended period of nutrient resorption (Schaberg et al., 2008, Hoch et al., 2003). Anthocyanin accumulation can occur in different leaf cell types (Merzlyak et al., 2008, Chalker-Scott, 1999), and across a range of leaf ages (Hackett, 2002). Distribution in vine leaves in this trial appeared to be on leaves young and old.

Vines with the most visible anthocyanin levels in this trial were those that received high rates of nitrogen at timings that made it available early in the season. There are a number of examples in the literature that indicate that a high carbon to nitrogen balance in leaves can lead to anthocyanin accumulation. High sugar to nitrogen ratios in grape tissue culture media will increase anthocyanin production, while greater nitrate availability will lead to a decrease (Pirie and Mullins, 1976, Do and Cormier, 1991b, Kumar and Sharma, 1999, Chimmad and Panchal, 1998). Treatments that decreased nitrogen availability following a high-nitrogen regime has been shown to also lead to an increase in leaf anthocyanin production in eucalypt seedlings (Close et al., 2000), while increasing leaf carbohydrate concentration in apple trees following fruit harvest also led to increasing leaf anthocyanin (Tartachnyk and Blanke, 2004). Both nutrient starvation and high levels of sugar were able to stimulate anthocyanin production in duckweed (Kumar and Sharma, 1999). The treatments in this trial where anthocyanin was observed all had increased canopy growth. The increased levels of leaf chlorophyll that resulted from higher nitrogen may have increased photosynthesis (discussed above), and it is possible that this, combined with increased leaf area, led to greater levels of carbohydrates in the leaves and vine. The timing of nitrogen addition in 2007-08 did not have an impact on leaf anthocyanin accumulation. However, leaf chlorophyll concentration measurements (Figure 4.2-8), and their correlation with nitrogen levels (Table 4.2-11), indicated that the leaf nitrogen concentration was likely to vary between timing treatments. Therefore, the results from these trials suggest that leaf anthocyanin production may be a sign of higher leaf carbohydrate concentration.

4.2e Conclusion

Nitrogen additions led to an increase in canopy growth, particularly when nitrogen was made available early in the season. This occurred when nitrogen was added prior to bloom, or as a result of nitrogen added in the preceding season. The impact of nitrogen added in the preceding season increased with later nitrogen additions. Leaf chlorophyll concentration was increased as nitrogen increased, and senescence could be delayed by nitrogen added at any stage following bloom until after veraison. These changes meant that nitrogen applications could increase canopy density, which may have implications for wine quality.

Nitrogen additions around veraison may be an effective way to delay senescence and increase nitrogen availability in the following season in cool climates, where post harvest nitrogen applications may be less successful as a result of decreased leaf health.

Leaf anthocyanin accumulation was linked to early nitrogen availability. With further research, this attribute has the potential to be used as an indicator of the vine nitrogen status late in the season.

4.3 Nitrogen addition to deficient grapevines (*Vitis vinifera* L.) cv Pinot Noir at different application timing and rates: Effects on yield, fruit development and fruit attributes

4.3a Introduction

The stimulation of grapevine growth by nitrogen application on deficient vines has been observed in many research papers (Bell and Robson, 1999, Candolfi-Vasconcelos et al., 1997, Conradie and Saayman, 1989, Grechi et al., 2007, Keller et al., 1998, Keller et al., 2001b), and confirmed in our own studies (section 4.2, p. 91). Changes in canopy density will impact fruit quality by changing the exposure of fruit to sunlight, with increasing sunlight generally leading to more phenolic compounds and anthocyanins in the grape skin, and reduced juice titratable acidity. Increases in nitrogen may also directly reduce the development of phenolic compounds (Pirie and Mullins, 1976), which can combine with the impact of increased canopy density to further reduce phenolics (Keller and Hradzina, 1998, Spayd et al., 2002).

Phenolic compounds have important roles to play as sun protection compounds and also in combating disease (Keller, 2010). Therefore, reductions in phenolics due to high nitrogen levels can leave fruit more vulnerable to sun damage and infections such as botrytis (*Botrytis cinerea*) and powdery mildew (*Uncinula necator*) (Keller et al., 2003). However, even more crucial may be the changes in microclimate within the canopy (Mundy and Beresford, 2007). Increasing canopy density can also increase disease risk further, due to the increase in humidity, as well as difficulties in reaching those areas when applying antifungal agrochemicals (English et al., 1989).

There can also be changes in sink:source relationships from alterations in the canopy. As well as having less leaves, those that are present on nitrogen deficient vines will have reduced photosynthetic capacity (Kumar et al., 2002, Vasconcelos et al., 2005), which may limit the ability of the vine to supply sugars to ripening fruit. Additional nitrogen supplied to vines with low nitrogen availability can lead to increases in leaf health or leaf area (Keller et al., 2001b, Vasconcelos et al., 2005). Conversely, active growing shoots are a resource sink that can compete with fruit for carbohydrates (Smart, 1991), and this can lead to a delay in sugar accumulation in the fruit. Excess availability of nitrogen that leads to delays in maturation has been observed in a range of trials (Delgado et al., 2004). Vine nitrogen availability can determine the level of nitrogen in the fruit, including the yeast assimilable nitrogen (YAN) (Treeby et al., 2000, Wade et al., 2004).. Musts with YAN levels that are below the amount required to fully ferment all available sugars are susceptible to have stuck or sluggish fermentations (Henschke and Jiranek, 1993), due to stressed yeast. This can lead to reduced wine quality by potentially leading to spoilage by other yeast or bacteria, or the production of excess hydrogen sulfide by yeast.

An addition of nitrogen can increase yields of deficient vines (Kliewer et al., 1991, Kliewer and Cook, 1974, Bell and Robson, 1999), through increased bud burst, fruitfulness and fruit set (Keller et al., 1998, Kliewer et al., 1991, Keller et al., 2001a, Spayd et al., 1993, Ewart and Kliewer, 1977). Under some situations, berry size may also be affected (Holzapfel and Treeby, 2007, Spayd et al., 1993). Excessive canopy growth may have the opposite effect due to shading of buds (Dry, 2000).

Vine nitrogen can vary greatly. In Tasmania, many vineyards are cultivated with a

permanent grass interrow, which can compete with the vines for nutrient availability (Larcheveque et al., 1998), and there is anecdotal evidence that nitrogen fertilisation is often avoided. Vines have been estimated to remove 3.9 kg N per tonne of harvested grape (Conradie and Saayman, 1989), and the supply for consecutive season's must either come from the soil, or be increased by fertilisation.

This trial was established to examine links between late season leaf health, vine growth and fruit attributes in a block of Pinot Noir under a permanent grass inter-row. An early part of the study determined that the late season leaf health in the block responded to the addition of nitrogen. A trial was set up to investigate the impact of nitrogen at moderate and high application rates and different fertilisation timing on the vine vegetative growth, fruit yield and attributes, and wine quality. Data from the trial will assist in determining the optimum management to maximise wine quality from vineyards with poor late season leaf health.

This chapter outlines the impacts of nitrogen additions on fruit yield and chemical attributes. The impact on leaf health and vine vegetative growth was investigated and is detailed in the preceding chapter, while effects on fermentation and wine quality are detailed in a later chapter.

4.3b Materials and Methods

Trials

Trials in this chapter include the nitrogen application and irrigation trial conducted in 2005-06, the nitrogen rate by timing trial, conducted in 2006-07 and 2007-08, and the nitrogen by exposure trial.

The nitrogen application and irrigation trial consisted of three rates of nitrogen (0, 17 and 51 g N/vine) and three irrigation rates (0, standard and high). Treatments were applied at veraison in the 2005-06 season. Full trial layout is described in the materials and methods section (p. 22).

The nitrogen rate by timing trial consisted in 2006-07 of three nitrogen rate (20, 35 and 50 g N/vine) applied at four dates (pre-bloom, post bloom, pre-veraison and post veraison), and an untreated control. In 2007-08 nitrogen was reapplied to all treatments except the 35 g N/vine treatment, which was also excluded from monitoring. Full trial layout is described in the materials and methods section (p. 22).

The nitrogen by exposure trial involved an application of nitrogen at a rate of 100 g N/vine pre-bloom in 2006-07 season. Fruit was then segregated into exposed fruit and shaded fruit, based on the location of bunches in the canopy. Full trial layout is described in the materials and methods section (p.23).

*Table 4.3-1 Analysis summary for trials in this chapter, including date carried out and reference page in the general materials and methods section. TSS - total soluble solids; TA - titratable acidity; YAN - yeast assimilable nitrogen. * - more information on this analysis below the table*

Trial	*Analysis*	*Date*	*Reference page*
Nitrogen by irrigation trial	Flowering date	14/12/06	33
	Yield and yield components	6/04/06 6/04/07	34
	Juice TSS, pH and TA	8/04/06 7/04/07	33
	YAN	12/05/06	34
Nitrogen rate by timing trial	Fruit set	2007-08	33
	Fallen berry analysis	27/03/07	31
	Veraison progression*	13/02/07 6/02/08	
	Yield and yield components	10/04/07 31/03/08	34
	Fruit chemistry	11/04/07 1/04/08	33
	YAN	23/04/07 15/04/08	34
	Berry tannin and anthocyanin concentration	06/04/08	34
	Botrytis infection level*	10/04/07 22/03/08 31/03/08	
Nitrogen by exposure trial	Fallen berry counts	27/03/07	31
	Shrivelled berry counts	12/04/07	31
	Rachis length measurement	16/04/07	31
	Fruit chemistry	12/04/07	33
	Berry tannin and anthocyanin concentration	12/02/08	34

Veraison progression

In the first year of the nitrogen application rate by date trial, estimates were made of percentage progression of veraison for each bunch on the monitor shoots. For analysis these percentages were converted into arcsines. It was also grouped into categories of 0-40%; 41-79% and 80-100%.

In the nitrogen application date by rate trial in 2007-08, all bunches were assessed. The percentage of veraison progression was judged to fall into one of the following categories: 0-9%; 10-20%; 21-40%; 41-59%; 60-79%; 80-90%; and 91-100% of berries on the bunch having commenced veraison.

Botrytis levels

In the 2006-07 season, botrytis bunches were counted and weighed during harvest.

These bunches were then discarded.

In 2007-08, botrytis bunches were removed at two stages. A pass was made on the 22nd March to remove botrytis bunches. All bunches with visible infection were weighed and counted, then discarded. This was to reduce the risk of botrytis infected berries being included in winemaking.

A second removal of bunches was carried out during the main harvesting of the fruit. Any bunches with visible infection were counted and discarded, but not weighed.

4.3c Results

Flowering date

Nitrogen addition in the veraison of 2006 led to earlier capfall in the following growing season (Table 4.3-2). No treatments affected the number of bunches per shoot.

*Table 4.3-2 Capfall percent on the 14th of Dec 2006 and bunch per shoot in response to nitrogen fertilisation. Arcsine transformations of percentages were used for ANOVAs. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Bunch 1 capfall (%)	Bunch 2 capfall (%)	Bunches per shoot
0	68a	55a	1.6
17	73ab	50a	1.4
51	85b	75b	1.6
Sig	*	*	n.s.

There was a change in the rate of capfall as a result of position of the shoot along the cane (Table 4.3-3). Basal shoots tended to have a delayed capfall, compared to shoots coming from the centre or end of canes. These shoots also had a reduced number of bunches per shoots, although not significantly different to the shoots from the tip of the cane. The most fruitful shoots came from the central region of the cane.

None of the changes in capfall progression resulting from treatments were to the same extent as the variability within vine resulting from shoot position along the cane (Table 4.3-3).

*Table 4.3-3 Capfall progress and bunch counts related to shoot position along the cane. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Shoot position	Bunch 1 capfall (%)	Bunch 2 capfall (%)	Bunches per shoot
Cane tip	85a	72a	1.50a
Mid cane	88a	65a	1.76b
Basal	58b	40b	1.38a
Sig	***	***	***

Fallen Berries

In 2006-07, it was noted that there was a large number of berries had fallen from bunches on vines where nitrogen was added prior to bloom. In the nitrogen timing by rate trial, higher fallen berry counts were associated with pre-bloom nitrogen additions (Table 4.3-4). Increasing rates of nitrogen did not alter the fallen berry counts (Table 4.3-5).

*Table 4.3-4 Fallen berry counts in response to the timing of nitrogen application in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

N date	*Fallen berry count*
Control	1.9a
Pre-bloom	9.8b
Post bloom	3.1a
Pre-veraison	2.4a
Post veraison	1.9a
Sig	***

*Table 4.3-5 Fallen berries prior to harvest in 2006-07 from vines treated with varying rates of nitrogen at veraison in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	*Fallen berry count*
0	1.9
20	3.8
35	4.9
50	4.2
Sig	n.s.

No fallen berry differences were observed in 2007-08. In fact, the phenomenon was not observed to be occurring in any treatments in this season.

The nitrogen addition by bunch exposure trial (where nitrogen was added at a rate of 100 g N/vine pre-bloom) was also observed to have a greater number of fallen berries in response to nitrogen addition (Table 4.3-6). The berries that fell were noticeably shrivelled in appearance, and this was further examined in the nitrogen addition by bunch exposure trial. The results indicated that nitrogen had a major impact on the degree of shrivel, while there was no impact from cluster exposure on the proportion of berries that shrivelled (Figure 4.3-1). Increased exposure did have a slight impact on reducing berry weight, both for shrivelled and non-shrivelled berries (Table 4.3-7).

*Table 4.3-6 Fallen berries as a result of a pre-bloom nitrogen application at 100 g N/vine, from vines in the nitrogen by exposure trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	*Fallen berry count*
0	1.9
100	9.0
Sig	***

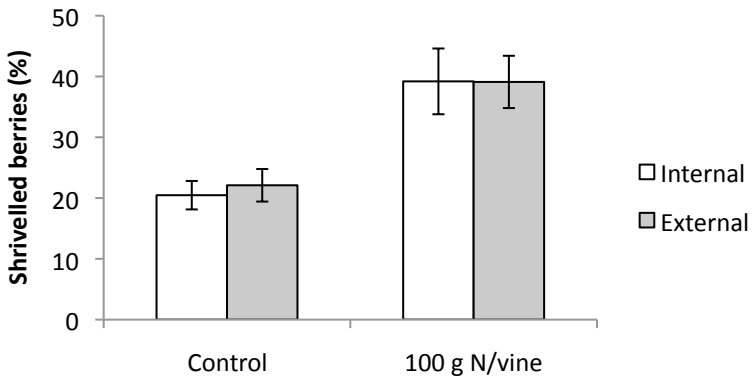

Figure 4.3-1 Impact of fertilisation with 100 g N/vine, and bunch position in the canopy on the degree of berry shrivel, assessed as the percentage of berries shrivelled at harvest, in 2006-07. Error bars represent standard error of the mean.

*Table 4.3-7 Impact of exposure and nitrogen addition on berry weights of shrivelled and non-shrivelled berries in 2006-07. 100 g N/vine was added to the fertilised treatments. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Non-shrivelled berry wt (g)*	*Shrivelled berry wt (g)*
Unfertilised	1.07	0.45
Fertilised	1.00	0.45
Sig	n.s.	n.s.
Internal	1.09b	0.48
External	0.98a	0.42
Sig	*	*

Fruit maturation progression

Veraison progression in 06-07 showed a significant response to timing of nitrogen addition, and a significant interaction effect between timing and rate (P=0.045) (Figure 4.3-2), Pre bloom additions appeared to accelerate development, and the effect increased as rate increased. Conversely, post bloom additions tended to decrease as rate increased however this decrease was not significant. Pre-veraison additions were mid way between these two, as was the control.

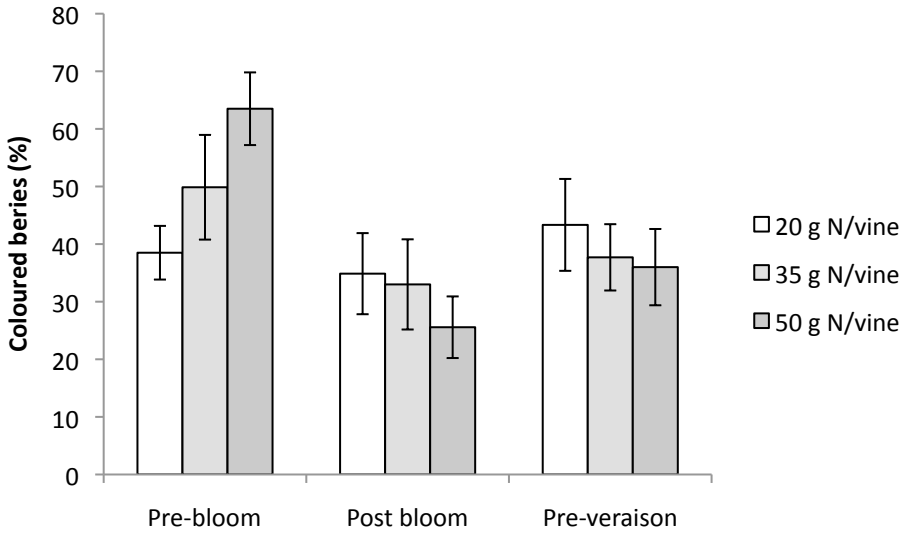

Figure 4.3-2 Impact of rate and date of application of nitrogen on the percentage of berries on basal bunches that had visible skin anthocyanin in 2006-07. Error bars represent standard error of the mean. Control average 37.3%.

Fruit from pre bloom applications were characterised by having a more bunches with over 80% of the berries having commenced veraison, while the post bloom application had a higher representation of bunches with less than 40% berries commenced veraison (Figure 4.3-3). Both pre veraison applications and the control had similar proportions in each segment.

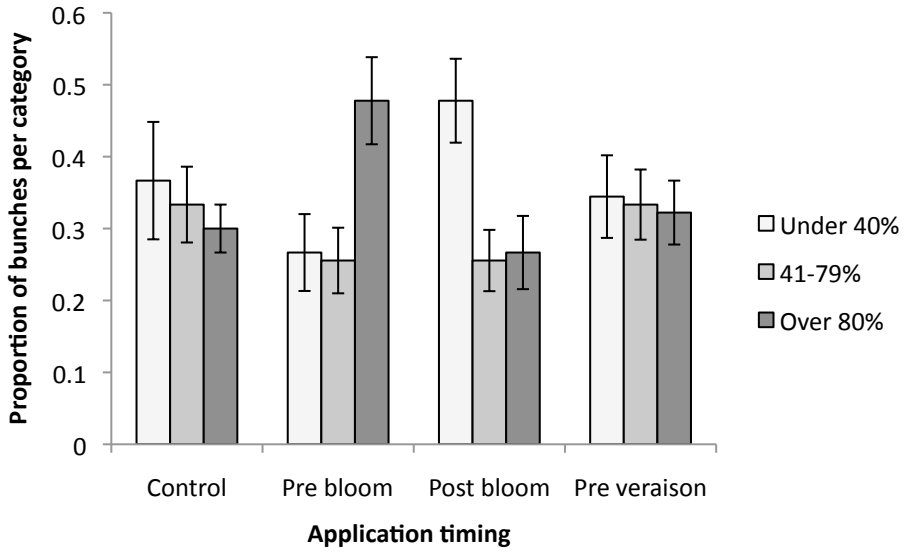

Figure 4.3-3 Impact of timing of nitrogen application on veraison progression in 2006-07. Bunch veraison development was separated into three categories – less than 40% berries coloured; 41-79% berries coloured; and 80% of berries coloured. Error bars represent standard error of the mean.

Post bloom nitrogen addition in 2007-08 led to a delayed veraison (Figure 4.3-4). This was indicated by an assessment of the percentage of bunches monitor shoots that had commenced veraison.

A survey of every bunch on all shoots of the monitor vines had better resolution. These indicated that the post bloom application resulted in a greater proportion of bunches

below 40% veraison, and less above 80% veraison compared to other dates (Figure 4.3-5).

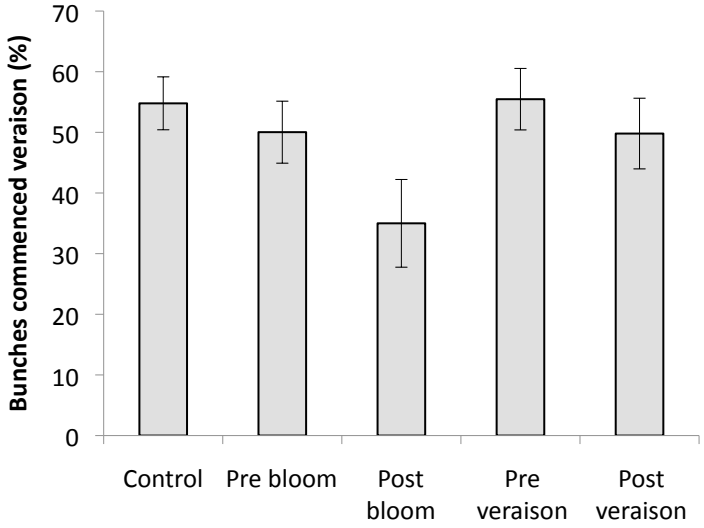

Figure 4.3-4 Impact of nitrogen application date on veraison commencement on monitor shoot basal bunches in 2007-08. Error bars represent standard error of the mean.

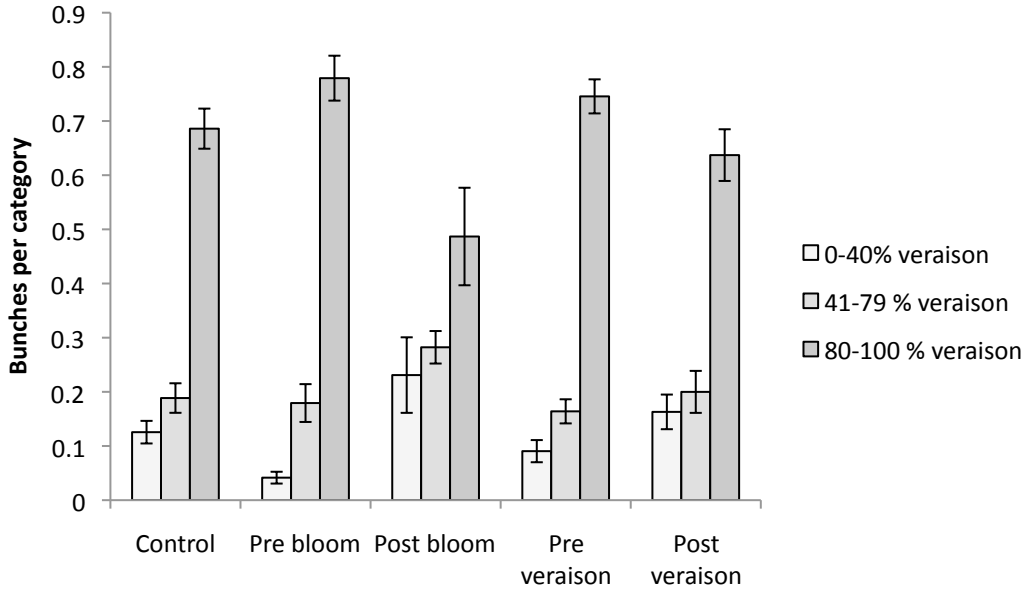

Figure 4.3-5 Impact of date of nitrogen application on veraison progression of all bunches on monitor vines in 2007-08. Bunch veraison development for each application date was separated into three categories – less than 40% berries coloured; 41-79% berries coloured; and 80% of berries coloured. Error bars represent standard error of the mean.

Yield and yield components

The nitrogen by irrigation trial had no significant effect on yield or any of the yield components measured in the year of its establishment (2006). In the same plots in the following season there were significant increases in bunch weight in response to nitrogen concentration, however there was no significant change in yield (Table 4.3-8.)

*Table 4.3-8 Yield components in 2006-07 in the nitrogen by irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Per vine yield (kg)	Bunch weight (g)	Bunch count	Berry weight (g)
0	4.0	110a	36.2	1.3
17	4.5	122b	37.1	1.3
51	4.5	113a	39.6	1.4
Sig	n.s.	**	n.s.	n.s.

In 2006-07, the nitrogen timing by rate trial showed no significant difference in neither yields, nor in any of the yield components, in response to either nitrogen timing or rate of application.

Regression analysis showed that yield was more strongly influenced by bunch count ($R^2 = 0.62$) than by bunch weight variation ($R^2 = 0.24$) in this block.

In 2007-08 there was an increase in the bunch count on fertilised vines, particularly in the 20g N/vine treatment level, as a result of more shoots per vine and a higher bunch count per vine (Table 4.3-9). There was a trend towards lower average berry weight in the 20 g N/vine treatments also, however this was not significant (P=0.057).

*Table 4.3-9 Yield components in the second year of the nitrogen application date by rate trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	Per vine yield total (kg)	Per vine yield clean fruit (kg)	Bunch weight (g)	Berry weight (g)	Bunches per vine	Shoots per vine	Bunches per shoot
Control	3.2a	3.1a	101	1.3	31.8a	26.6	1.2
20	4.1b	3.7b	106	1.2	39.3b	29.3	1.3
50	3.6ab	3.0a	102	1.3	35.9b	29.0	1.2
Sig	**	**	n.s.	n.s.	**	n.s.	n.s.

Botrytis infection in 2007-08 increased the differences in fruit yields, following removal of diseased fruit. Rejection of botrytised bunches gave the 20 g N/vine treatment a significantly higher bunch count.

Rachis measurements and fruit set

Measurements of rachis lengths, pedicel counts and pedicel branches were undertaken during the 2006-07 season to assess the relative and combined impact of nitrogen and exposure on individual bunches.

Results showed an increase in the number of rachis branches as a result of increasing exposure. There was no difference in bunch length. There was an increase in the number of pedicels per branch as a result of nitrogen addition, indicating that pre bloom nitrogen may have increased fruit set in this situation (Table 4.3-10).

*Table 4.3-10 Rachis measurements of bunches harvested from fertilised and unfertilised vines and segregated into different exposure levels. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Rachis length*	*Number of branches*	*Pedicels per branch*	*Pedicels per mm of rachis*
Unfertilised	89	15.1	8.4	1.4
Fertilised	91	15.3	10.6	2.0
Sig	n.s.	n.s.	***	*
Internal	91	14.7	9.2	1.5
External	89	15.7	9.8	2.0
Sig	n.s.	*	n.s.	n.s.

Fruit set was specifically measured in 2007-08, and no differences were found. There was also no berry shrivel observed in this season.

Fruit chemistry

Nitrogen rates of 17 g N/vine added at veraison in the 2005-06 season led to a decrease in total soluble solids (TSS) (Table 4.3-11). There were no significant differences in pH or TA, nor any significant interactions.

*Table 4.3-11 Response of nitrogen addition at veraison on basic fruit chemical parameters, in the nitrogen by irrigation trial in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	*TSS (Brix)*	*pH*	*TA (g/L)*
0	23.9ab	3.41	7.5
17	23.2a	3.37	7.4
51	24.1b	3.39	7.4
Sig	*	n.s.	n.s.

Irrigation removal had no effect on TSS, however there was a TSS decreases from supplemental irrigation. No irrigation treatments led to any significant changes to pH or TA (Table 4.3-12), and there were no significant interactions.

*Table 4.3-12 Response from altered irrigation rates post veraison on basic fruit chemical parameters, in the nitrogen by irrigation trial in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Irrigation	*TSS (Brix)*	*pH*	*TA (g/L)*
Nil	24.1a	3.40	7.4
Norm	24.1a	3.40	7.4
High	23.1b	3.36	7.5
Sig	**	n.s.	n.s.

In the nitrogen and irrigation trial in the subsequent season, there were increases in TSS, TA and pH in juice from vines that had received 51 g N/vine (Table 4.3-13) in 2005-06. Irrigation removal had negligible impact on juice chemistry (Table 4.3-14). There were no significant interactions.

*Table 4.3-13 Juice chemistry responses to nitrogen rate, from the nitrogen and irrigation trial in 2006-07, following nitrogen addition in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	TSS (Brix)	pH	TA (g/L))
0	23.1a	3.19a	6.8a
17	23.7a	3.21a	6.9a
51	24.6b	3.27b	7.2b
Sig	**	**	*

*Table 4.3-14 Juice chemistry responses to irrigation deficit in the previous season rate, from the nitrogen and irrigation trial in 2006-07, following nitrogen addition in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Irrigation rate	TSS (Brix)	pH	TA (g/L)
Nil	24.0	3.21	7.1b
Standard	23.5	3.23	6.8a
Sig	n.s.	n.s.	*

Total soluble solids were increased in the nitrogen timing by rate trial 2006-07 as a result of nitrogen addition (Table 4.3-15). Earlier additions led to higher TSS, with the post veraison application not significantly different to the control (Table 4.3-16). There were no differences in TSS in the subsequent season, and no significant interactions.

Juice pH did not vary in 2006-07 as a result of any treatment in the nitrogen timing by rate trial, although titratable acidity did increase as the rate of nitrogen added was increased (Table 4.3-15). There was also a notable spike in the TA of the post bloom treated vines (Table 4.3-16).

*Table 4.3-15 Basic fruit chemistry response to nitrogen rate in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	TSS (Brix)	pH	TA (g/L)
0	23.9a	3.39	5.8a
20	24.7bc	3.43	5.8*a*
35	24.5ab	3.41	6.2*b*
50	25.1c	3.44	6.3*b*
Sig	*	n.s.	**

*Table 4.3-16 Basic fruit chemistry responses to timing of nitrogen application in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	TSS (Brix)	pH	TA (g/L)
Control	23.9a	3.39	5.8a
PrB	25.7c	3.45	6.0a
PoB	24.8b	3.43	6.6b
PrV	24.6ab	3.43	5.8a
PoV	24.1a	3.39	5.9a
Sig	***	n.s.	***

Total soluble solids and pH were clearly higher in the vines that had received 100 g N/vine, indicating increased fruit maturity (Table 4.3-17). TA levels did not change. The fruit from these vines had higher Brix levels and pH and lower TA in response to increased fruit exposure (Table 4.3-18), indicating that increasing exposure was also leading to more mature fruit. There were no significant interaction effects, however.

*Table 4.3-17 Brix, TA and pH levels of harvested fruit in response to 100 g N/vine applied pre bloom in 2006-07 season. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	***TSS (Brix)***	***pH***	***TA (g/L)***
0 g N/vine	22.9a	3.46a	6.1
100 g N/vine	24.4b	3.58b	6.3
Sig	***	**	n.s.

*Table 4.3-18 Brix, TA and pH levels in response to fruit exposure in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Fruit position	***TSS (Brix)***	***pH***	***TA (g/L)***
Internal	23.3a	3.48a	6.7a
External	23.9b	3.56b	5.7b
Sig	**	**	***

Juice pH was higher in the 50 g N/vine treatment in the nitrogen timing by rate in 2007-08 in pre-harvest samples. The control vine pH increased however, and by the time of harvest both control and 50 g treated vines were higher than the 20 g treated vines (Table 4.3-19). TA was increased by fertilisation, and tended to increase as the rate increased. There were no significant rate effects (although the post bloom treatment trended higher than the others) (Table 4.3-20), and no significant interactions.

*Table 4.3-19 Basic fruit chemistry for different rates of nitrogen application in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	***TSS (Brix)***	***pH***	***TA (g/L)***
0	24.4	3.49ab	5.8a
20	24.7	3.44a	6.1ab
50	24.5	3.50b	6.3b
Sig	n.s.	*	*

*Table 4.3-20 Basic fruit chemistry for different application dates in 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Application timing	***TSS (Brix)***	***pH***	***TA (g/L)***
Control	24.4	3.49	5.8
PrB	24.5	3.45	6.0
PoB	24.3	3.46	6.5
PrV	24.5	3.47	6.1
PoV	24.9	3.51	6.3
Sig	n.s.	n.s.	n.s.

In 2005-06, the first year of the nitrogen by irrigation trial, YAN was higher in the unirrigated vines (Table 4.3-21). YAN was decreased by application of 17 g N/vine the middle rate of nitrogen addition. There was no difference between the YAN levels of the control and high nitrogen rate vines.

*Table 4.3-21 Yeast assimilable nitrogen in response to nitrogen addition at veraison and irrigation rate after veraison in 2005-06. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Rate*	*YAN (mg/L)*	*NH_3:Amino Acid*
Nitrogen	0	162.7b	0.26
(g N/vine)	17	135.6a	0.25
	51	167.5b	0.26
	Sig	**	n.s.
Irrigation	Nil	178.8b	0.28
	Normal	150.0a	0.25
	High	137.1a	0.25
	Sig	***	n.s.

YAN was linked to maturity, with a significant correlation between YAN and TSS (R^2=0.62) in the irrigation and nitrogen trial in 2005-06.

In the following season of the nitrogen by irrigation trial, YAN was increased in the vines where nitrogen application had been higher (Table 4.3-22), while there was no impact from irrigation treatments (data not presented).

*Table 4.3-22 YAN and component ratios in 2006-07 in the nitrogen by irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	*YAN (mg/L)*	*NH3:Amino Acid*
0	148.5a	0.18
17	156.7a	0.19
51	244.4b	0.25
Sig	***	***

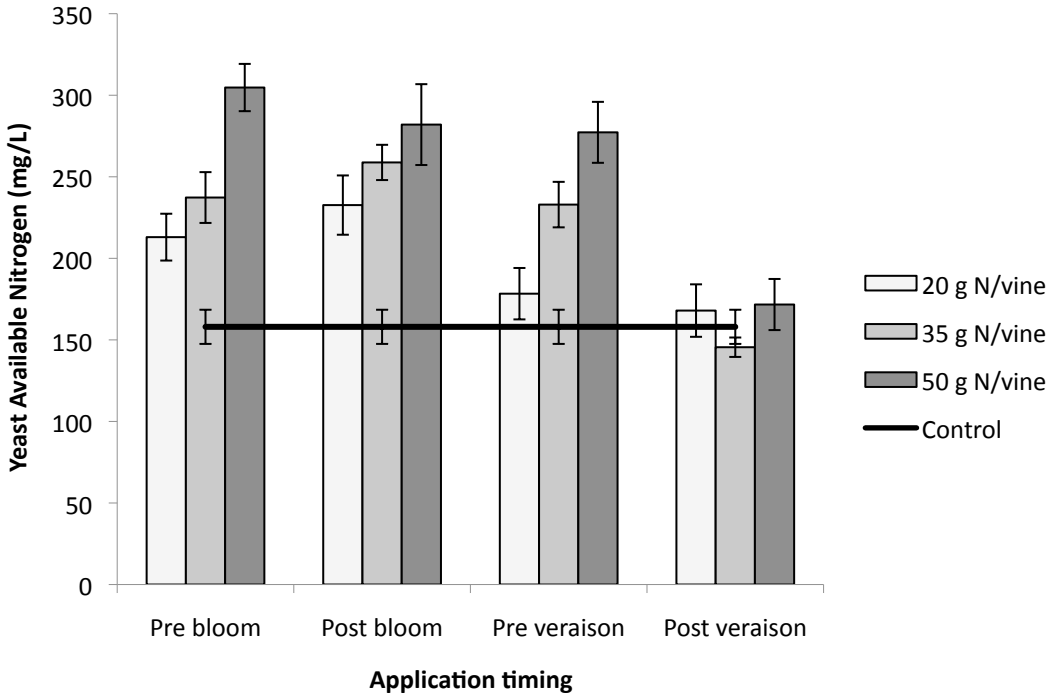

Figure 4.3-6 Yeast assimilable nitrogen in must in 2006-07 for four different application dates at three rates (20, 35 and 50 g N/vine), and an untreated control. Error bars represent standard error of the mean.

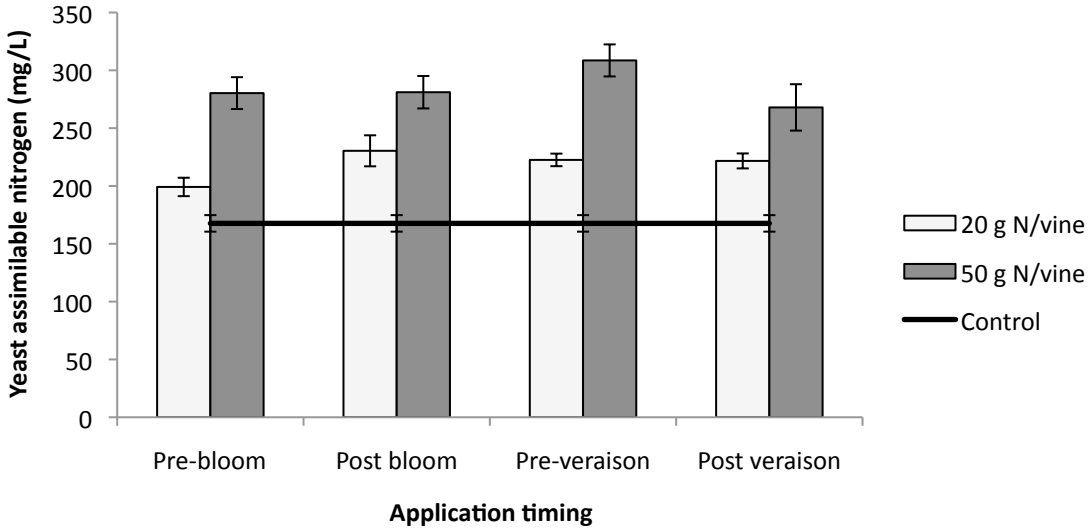

Figure 4.3-7 Yeast assimilable nitrogen for each treatment in 2007-08 for four different application dates at two rates (20 and 50 g N/vine), and an untreated control. Error bars represent standard error of the mean.

Increased nitrogen rates led to an increase in juice YAN in both years of the nitrogen rate by date trial (Figure 4.3-6, Figure 4.3-7).

In 2006-07, pre-bloom and post bloom additions were the most effective at raising YAN levels, while post veraison additions did not increase YAN above the control vines (Figure 4.3-6). There was a significant interaction effect between rate and timing in 2006-07 (P=0.048), with significant increases in YAN in response to rate in all timings except

the post veraison application. In 2007-08 there were no significant differences in YAN content as a result of different application dates (Figure 4.3-7).

Nitrogen applications also altered the proportions of nitrogen in the ammonium form compared to nitrogen in amino acids. Pre-bloom and post bloom applications in both seasons increased the proportion of nitrogen in the must that was in the ammonium form (Figure 4.3-8, Figure 4.3-9). In 2006-07, increasing the rate for the pre-bloom and pre-veraison applications also led to a proportional increase in ammonium (Figure 4.3-8). This was also apparent with pre-bloom additions in 2007-08 (Figure 4.3-9), and there was a significant interaction between timing and rate in that season (P=0.32).

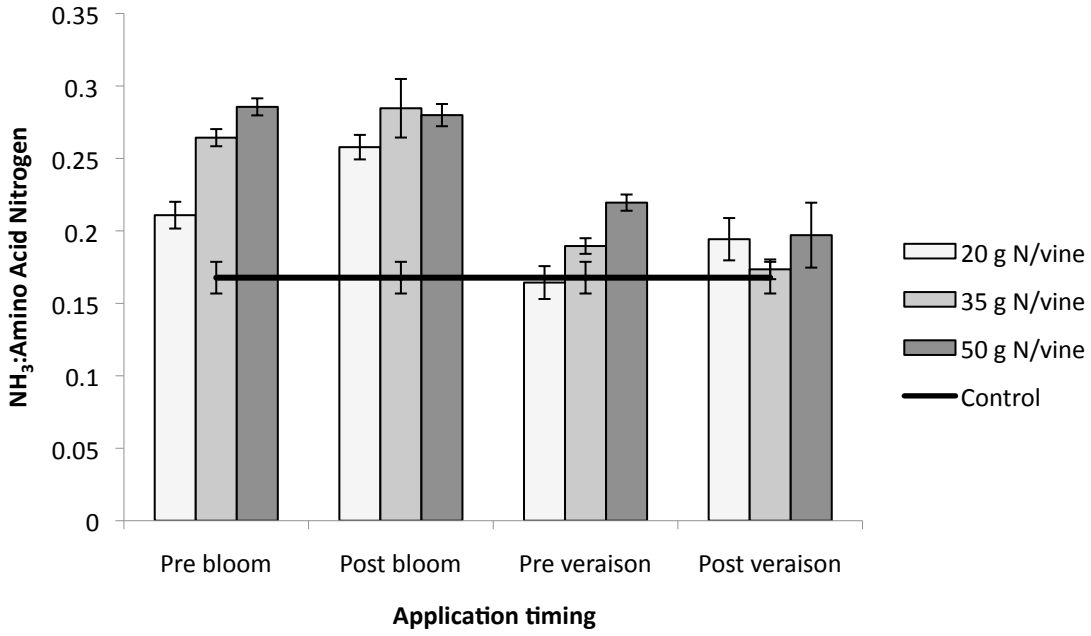

Figure 4.3-8 Ammonia nitrogen to Free Amino Acid nitrogen ratios in different nitrogen timing and rates of application in 2006-07. Error bars represent standard error of the mean.

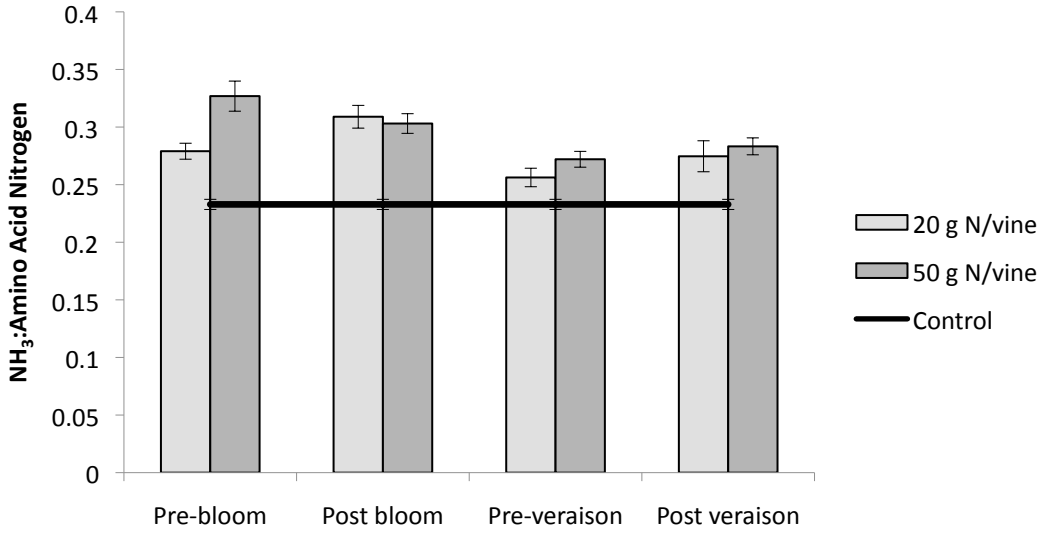

Figure 4.3-9 Ammonia nitrogen to free amino acid nitrogen rations in different nitrogen timing and rates of application in 2007-08. Error bars represent standard error of the mean.

In 2007-08, YAN and leaf nitrogen content (Table 4.2-9, Table 4.2-10) were better

correlated with veraison measurements of leaf nitrogen than with bloom measurements (Table 4.3-23).

Regression analysis of the control vines alone indicates that the bloom leaf nitrogen measurements were better correlated to YAN than veraison measurements, which were not significantly correlated to YAN (Table 4.3-23).

*Table 4.3-23 R^2 values for regressions between leaf nitrogen concentration in the nitrogen rate by timing trial at different dates in the season and must YAN. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Leaf sampling date	All vines	Control vines only
Bloom	0.39	0.33
Sig	***	*
Veraison	0.68	0.21
Sig	***	n.s.

Berry extract chemistry

Berry homogenate extracts from 2007-08 showed no significant differences between treatments in the nitrogen timing by rate trials, although Iland phenol measures were almost significantly lower in treatments receiving nitrogen (P=0.051) (Table 4.3-24).

*Table 4.3-24 Berry extract analysis from nitrogen timing by rate trials, 2007-08. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen treatment	Berry Tannins (mg/g homogenate)	Iland phenols AU	Anthocyanin (mg/g homogenate)
Control	8.2	1.13	0.66
All N combined	8.0	1.10	0.67
Sig	n.s.	n.s. (P=0.051)	n.s.

The nitrogen by exposure trial did show a significant interaction effect in the analysis of berry extract chemistry (P=006). Fertilised, external bunches had significantly less phenolics than all other treatments. There were no significant differences between any o the other treatments (Figure 4.3-10).

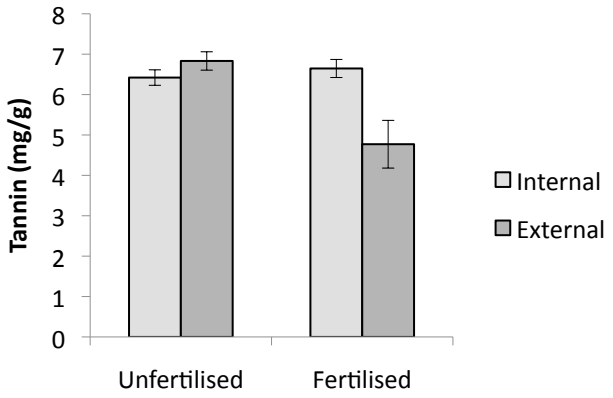

Figure 4.3-10 Berry tannins (mg per gram of homogenate) in fruit extracts from fruit off exposed or shaded sections of both unfertilised vines and vines fertilised with 100 g N/vine pre-bloom in 2006-07. Error bars represent standard error of the mean.

There were no significant differences in anthocyanin concentration in the berry extract

from either nitrogen addition or the level of fruit exposure (data not presented).

Botrytis levels

The incidence of botrytis infections was not significantly different between treatments in 2005-06 or 2006-07. In 2007-08 there was large differences between trials (Table 4.3-25, Table 4.3-26). The number of bunches that were affected by botrytis rose significantly as nitrogen rate increased. Pre-bloom applications induced more botrytis than other applications, with post veraison applications better than other timings (Table 4.3-26).

*Table 4.3-25 Botrytis incidence in response to nitrogen application rate in 2007-08. Infection was assessed at two stages – a pre-harvest disease thinning (bunches weighed), and again at harvest (bunches counted). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen rate (g N/vine)	***Botrytis-infected bunches (total per vine)***	***Pre harvest botrytis thinning fruit removed (g)***
0	1.1a	261a
20	4.2b	982b
50	6.8c	1420c
Sig	***	**

*Table 4.3-26 Botrytis incidence levels in response to date of application in 2007-08. Infection was assessed at two stages – a pre-harvest disease thinning (bunches weighed), and again at harvest (bunches counted). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen application timing	***Botrytis-infected bunches (total per vine)***	***Pre harvest botrytis thinning fruit removed (g)***
Control	1.1a	261a
Pre-bloom	8.2c	1857d
Post bloom	4.8b	1054c
Pre-veraison	5.3bc	1182c
Post veraison	3.4ab	711b
Sig	*	**

4.3d Discussion

Fruit set and yield

The increase in pedicels per bunch evident in the 2006-07 season as a result of pre bloom nitrogen fertilisation (Table 4.3-10) indicate that there was a decrease in flower mortality and abscission in response to increased nitrogen availability, since the nitrogen application was too late to have had an impact on flower number (May, 2000). A range of factors, including plant hormone levels and weather conditions during flowering, can influence fruit set, and stress around flowering will often reduce fruit set (Keller, 2010). The internal levels of a number of compounds with hormonal activity have been linked to fruit set, including ethylene (Bessis et al., 2000), auxins (Bessis et al., 2000), and polyamines. Nitrogen availability may influence the levels of many of these compounds. Both overall quantity and the form of polyamines present will vary in response to nitrogen stress. At fruit set a nitrogen deficiency can lead to more bound diaminopropane (Geny and Broquedis, 2002), and at anthesis higher free putrescine in the flowers.

Improved nitrogen status has been shown to increase fruit set of grapevines in other

trials (Conradie, 2004, Keller et al., 1998), and increased fruit set has also been linked to greater leaf area at bloom (Winkler, 1958). Bell and Robson (Bell and Robson, 1999) report that nitrogen additions to deficient vines led to an increase in berries per bunch, which may also have been a result of improved fruit set. However, excessive nitrogen can reduce fruit set (Winkler, 1970), as can high levels of shading due to increased canopy density (Dry, 2000). The lack of any change in fruit set in the following season in response to nitrogen indicates the impact of seasonal differences on fruit set, which can be large (Jackson, 1994, Vasconcelos et al., 2009). Cool climates and wet conditions can lead to reductions in fruit set (Vasconcelos et al., 2009). Nitrogen availability is also seasonally variable, responding to soil moisture and temperature (Jackson et al., 2008, Knoepp and Swank, 2002). If the second season nitrogen availability was greater than in the first, this may have been enough to remove the impact of low vine nitrogen status on fruit set that was implied by the first season pedicel counts. The second season showed an increased level of vine growth even in control vines, with higher overall pruning weights and cane weights (Table 4.2-23, Table 4.2-24) compared to the preceding season, and bloom time lamina analysis in 2007-08 showed only a marginal difference in nitrogen concentration between the control vines and those that had received nitrogen the preceding season (Table 4.2-10). These observations may be evidence of an increase in the availability of soil nitrogen early in the season of 2007-08.

The increase in fruit per bunch may have been the cause of the increase in berries that detached from the bunch during ripening, and the berry shrivelling. The bunches that exhibited the highest degree of berries falling from the bunch received early nitrogen (Table 4.3-4), which also were those that had higher numbers of pedicels per bunch, with no increase in rachis length (Table 4.3-10). It was clear from the lack of significant differences between exposed bunches and shaded bunches that it was not associated with increased exposure (Figure 4.3-1), so was not an impact of higher berry temperatures or other exposure related effects. There was no indication of fallen berries in the 2007-08 season, indicating the importance of seasonal effects on vine growth. No published studies were found where the same symptoms were described. One recent paper on the causes of berry shrivel provides four common factors that may lead to shrivel: dehydration; sunburn, bunch stem necrosis; and sugar accumulation disorder (Krasnow et al., 2010). The authors provide some symptoms of each of the four causes listed, however none match those seen in this trial. Another study noted that rachis nitrogen was elevated in one instance where shrivel was observed, however the berries in their study were not fully coloured and had low sugar levels (Smith et al., 2004), therefore this is probably not a related form of shrivel.

Yield was significantly increased by nitrogen application in 2007-08, associated with a higher bunch count per vine following the application of 20 g N/vine (Table 4.3-9), and higher shoot numbers per vine as a result of increased bud burst percentage. Yields from 50 g N/vine treatments were not significantly different to the control vines, in spite of increased bud burst (Table 4.2-15). The increase on the vines receiving 20 g N/vine appears to be from a combination of non-significant increases in bunch weight and bunches per vine (Table 4.3-9). When combined they resulted in a significant increase in fruit yield.

Flower development and maturation progression

Nitrogen applications in 2005-06 in the irrigation by nitrogen trial led to earlier flower development in 2006-07 (Table 4.3-2). This mirrors the result seen in the early season shoot development measurements of the same treatments, where vine average E-L stage pre bloom was also increased by nitrogen addition (Table 4.2-17). Vine shoot elongation

is linked to flowering (Vasconcelos et al., 2009), and the reduced shoot growth may have a direct impact on the flowering date. It has been suggested that the onset of flowering is related to the availability of carbohydrates; therefore shoots that have more mature leaves early may then have a correspondingly early flowering. The increased shoot elongation that was observed in this trial was associated with an increase in mature leaves prior to flowering.

The percentage of capfall also increased in response to nitrogen addition in the second year of the nitrogen timing by rate trial. All timings, with the exception of the post veraison application, led to an increased number of shoots with more developed flowering. The post veraison application had a response more closely aligned with the controls, even though it had similar shoot growth. Early season shoot growth in the timing by rate trial followed a similar pattern when comparing control vines to all nitrogen treated vines (Figure 4.2-13).

Veraison development in vines that received nitrogen pre bloom in 2006-07 increased in response to the rate of nitrogen addition, however no other timing showed this response (Figure 4.3-2). This effect was also seen in response to the highest rate of nitrogen addition in the preceding season. The vines with increased early nitrogen availability had more vegetative growth than other treatments, and this may have the effect of increasing sugar levels through an increased photosynthetic ability, or increased water stress from a higher leaf area.

Post bloom nitrogen applications appeared to lead to some delays in the onset and development of veraison. In the 2006-07 season, these differences were not significant, however in the 2007-08 season there were significantly more bunches with less than 40% veraison and less bunches with more than 80% veraison on vines that had received post bloom nitrogen. Whether this effect is a result of changes in the vine hormone levels, or changes in resources is unclear. Nitrogen will stimulate shoot growth, and in the initial stages of growth the shoots are a sink for vine resources. As such they can compete with the developing fruit for sugars. Sugar levels can stimulate the development of anthocyanins in the fruit, which will influence the development of veraison. The hormonal controls of veraison are not well understood, however they are certainly very important in regulating this process (Wheeler et al., 2009).

Botrytis infection

The level of botrytis infection was increased by nitrogen addition in the final year of the trial (Table 4.3-25, Figure 4.2-13, Figure 4.2-14), particularly when the nitrogen was applied pre bloom. Conradie (2001) observed a higher level of botrytis when nitrogen was applied at bud break, compared to a split application or a post harvest application. Christensen (1994) noted that increasing the application rate of nitrogen led to higher levels of botrytis, however they report a trend towards higher infection from veraison application timing. Nitrogen addition has also increased botrytis infection (Keller et al., 2001a) and powdery mildew levels (Keller et al., 2003) in other trials. Stilbenes, a group of phenolic compounds, are used by the plant to combat fungal infection (Gabler et al., 2003, Keller et al., 2003). Phenolic compound concentration in the fruit (Table 4.3-24, Figure 4.3-10) and in the wine (Figure 4.4-3) was reduced in response to high nitrogen rate, and although stilbenes were not specifically measured in this trial it is possible that their levels were reduced in response to nitrogen addition. However, it is probable that much of the link reported in this trial between nitrogen and botrytis susceptibility is due to modifications in canopy microclimate. This was also suggested by the authors of a study correlating YAN to botrytis infection in Sauvignon Blanc, which found that, while there was a positive correlation between YAN and botrytis levels, there was no evidence

to support the theory that YAN levels or nitrogen concentration were directly causing the increased disease (Mundy and Beresford, 2007). Botrytis infection has been reported to increase in response to higher canopy densities (English et al., 1989). Reduced exposure to light (particularly UV light) will lead to lower phenolic concentration (Adrian et al., 2000, Steel and Keller, 2000), and reduced air flow will increase the potential for infection by impeding spray penetration (Zoecklein et al., 1992) and slowing down the drying of fruit (English et al., 1989, Thomas et al., 1988). Assessment of botrytis levels on fruit from vines that spanned a range of vigour levels indicated that increasing canopy density is not the only factor that can lead to higher botrytis (Table 3.3-21, Table 3.3-22).

The greater impact of pre-bloom nitrogen additions also indicates that there may be other factors influencing the degree of infection, since these vines had similar levels of canopy growth. Conradie (Conradie, 2004) counselled against nitrogen applications prior to fruit set because of the potential for increased botrytis, with which the results of this trial agree. While excessive nitrogen may increase botrytis levels, canopy management to increase exposure and air flow may reduce the possibility of this occurring (English et al., 1989, Smart, 1991).

Fruit chemistry

Pre-bloom nitrogen additions in the nitrogen rate by date and nitrogen by exposure trials in 2006-07 led to higher berry soluble solids at harvest, increasing with higher nitrogen rates (Table 4.3-16, Table 4.3-17). Treatments that received nitrogen as part of the nitrogen by irrigation trial similarly led to increased Brix at harvest with treatments that would have increased nitrogen early in the season had a similar impact on the fruit Brix levels (Table 4.3-13). Nitrogen has been found to have a variety of impacts on fruit maturation, with some researchers seeing sugar levels increase (Treeby et al., 2000), some seeing a decrease (Conradie, 2001, Delas et al., 1991, Spayd et al., 1994), and some observing no change (Vasconcelos et al., 2005). One nitrogen application study observed delays in ripening in the early stages of development however these differences were less apparent by harvest (Keller et al., 1998). In vines where the production of sugars by the leaves is a limiting factor on the rate of ripening, nitrogen would be expected to increase sugar levels, since it can increase the number, size and photosynthetic capacity of leaves. In situations where photosynthetic production of carbohydrates is sufficient to meet the demands of the ripening fruit, an increase in the ability of the leaves to photosynthesise would have no net advantage, and the overstimulation of shoot growth may lead to competition for resources that can delay ripening. The results from 2006-07 may be indicative of a canopy that was struggling to supply the carbohydrate load required for ripening.

Increases in titratable acidity (TA) are often seen in response to nitrogen fertilisation (Bell and Henschke, 2005), and an increase in TA was clearly observed in response to nitrogen fertiliser rate in 2006-07, although there were no significant differences in TA in 2007-08. The timing of fertiliser addition also influenced the TA of the juice. Post bloom applications led to significantly higher TA in 2006-07 compared to all other dates, and there was a non-significant trend towards the highest level in 2007-08. This may be a reflection of the delayed maturity of these vines, as observed in veraison progression (Figure 4.3-4), however juice Brix were not reduced by the time of harvest. The greater canopy density of vines that were fertilised post bloom may also cause the increase. Greater shading of the fruit will increase malic acid, a major component of TA, since it is degraded more rapidly in warmer conditions (Jackson, 1994). While pre-bloom applications in 2006-07 did lead to increased canopy growth, this treatment suffered

from a decline in late season leaf health, compared to the post bloom applications. The fruit on the vines that received pre bloom nitrogen was also riper than other treatments, which would have further lowered TA.

Yeast assimilable nitrogen levels in the first nitrogen trial (Table 4.3-21) showed a result that is counter to most other results in literature, and indeed counter to most of the other results in this trial. In general, YAN increases with increasing nitrogen addition (Bell and Henschke, 2005). In 2005-06, adding 17 g N/vine at veraison decreased YAN in the must (Table 4.3-21), compared to both the control and the higher rate of nitrogen. Leaf chlorophyll analysis in other seasons indicated that senescence processes have begun by veraison, particularly where no extra nitrogen has been added (Figure 4.2-8). Senescence involves a large remobilisation of nitrogen from proteins in the leaf, particularly rubisco (Feller et al., 2008, Hörtensteiner and Feller, 2002), which is available for the fruit to then use to increase its nitrogen content. Nitrogen addition at both 17 g N/vine and 51 g N/vine had the effect of delaying leaf senescence processes, as assessed by leaf abscission (Figure 4.2-1) and this would have reduced the amount of remobilised nitrogen available to the fruit from the leaves. Post veraison there is little to no flow into the berry from the xylem (Bondada et al., 2005, Chatelet et al., 2008, Greenspan et al., 1994). Nitrogen taken up by the roots is transported in the xylem (Wermelinger, 1991), and would be first available to the leaves. Leaves will respond to increasing nitrogen availability by producing more Rubisco, and other proteins used in the leaf. Excess nitrogen may then be loaded into the phloem for distribution around other parts of the vine, including the fruit. It is proposed that the nitrogen levels in vines that received nitrogen at 51 g N/vine were enough to supply the immediate needs of the leaves, and therefore allow some nitrogen to be made available to the fruit. The application of 17 g N/vine may not have increased vine nitrogen enough to allow this to occur. In this way, the fruit were denied this source of nitrogen and also the nitrogen remobilised from senescence.

Nitrogen accumulation by the fruit occurs in two phases, one prior to the grapes reaching pea size, and the second starting at veraison (Wermelinger, 1991). In the final stages of ripening, nitrogen uptake will reduce, and in some cases may actually decline (Bell and Henschke, 2005). One exception to this is the amino acid proline, which will continue to increase through ripening, however proline is not assimilated by yeast under normal fermentation conditions (Ough and Stashak, 1974). Also, Pinot Noir is not a major accumulator of proline (Spayd and Andersen-Bagge, 1996). Pre-bloom and post bloom nitrogen additions had the most impact on berry nitrogen concentration in 2006-07, probably because these dates allowed nitrogen to enter the vine in time for the first period of uptake. The pre-veraison application of nitrogen made nitrogen available for the second uptake period. The post veraison application did not lead to any increase in YAN, possibly because the nitrogen was not made available in time for the major uptake periods of the fruit.

YAN in the following year of the timing by rate trial showed no significant differences between timings on the total YAN levels, although YAN was increased as the rate of nitrogen increased. Leaf nutrient and chlorophyll analysis indicated that there was substantial nitrogen made available to the vine from stored nitrogen absorbed in the previous season (Table 4.2-9). It is probable that nitrogen remobilised from these stores also increased nitrogen availability to the fruit in the initial growth berry phase, when substantial uptake will occur (Wermelinger, 1991), leading to all application timings having a similar impact on total YAN.

While the total YAN concentration may not have changed in response to fertiliser timing, the proportion of ammonium nitrogen to free amino nitrogen did change. In

2006-07, the highest proportion of ammonium in the must came from pre and post bloom applications of nitrogen. Post veraison applications had an ammonium:amino acid ratio that was not significantly different to the control, however the YAN was significantly increased. In 2007-08, it was again the pre and post bloom applications that were proportionally higher in ammonium. Ammonium levels are highest prior to veraison, and then decrease during ripening, while amino acids may continue to accumulate (Bell and Henschke, 2005). The two different nitrogen sources can impact fermentations in different ways. Ammonium is one of the most preferred nitrogen sources for yeast (Bell and Henschke, 2005). When there are high ammonium levels uptake of other less preferred sources, which includes many amino acids, will be suppressed, however the total YAN levels found in this trial would not be high enough to trigger this response. Yeast metabolism of different amino acids can alter the levels of esters in the wine (Guitart et al., 1999), which are important compounds that can contribute to wine aroma. Whether the small differences found here alter either the fermentation or the wine quality significantly is not known.

Nitrogen availability decreased the grape tannin concentration in the nitrogen by exposure trial, as has been reported by other researchers (Keller and Hradzina, 1998). This effect was only apparent on the exposed fruit of nitrogen treated vines, and not on the shaded fruit. Excessive light has been shown to reduce phenolic content in cases of extreme exposure (Bergqvist et al., 2001), however excessive exposure is considered unlikely to have occurred in these trials. The exacerbating influence of light (Figure 4.3-10) has not previously been reported in research, and deserves more attention. In most research, light levels lead to increased fruit phenolic production.

There were no significant decreases in phenolics or tannins observed in the fruit extracts in 2007-08 (Table 4.3-24) from the nitrogen timing by rate trial. There were also no significant differences in fruit brix levels at harvest (Table 4.3-19). Therefore, the results of the berry tannin extraction indicate that reduction of fruit tannins by nitrogen addition are minor at the levels of nitrogen used in this trial.

4.3e Conclusion

Nitrogen addition increased fruit yield as a result of higher bud burst leading to more shoots. It appeared that nitrogen fertilisation could lead to an increase in fruit set, however the potential for this to be influenced by other environmental factors means that nitrogen fertiliser addition may not always influence set. Increasing bud burst from higher rates of nitrogen may be offset by fewer nodes per vine (Table 3.2-5, Table 3.2-6, Table 4.2-15, Table 4.2-16) and also by an increase in botrytis infection levels.

Changes in fruit chemistry in response to nitrogen availability were also influenced by seasonal impacts. In 2006-07 additional nitrogen increased the rate of ripening of the fruit, however TSS did not vary in response to nitrogen in the following season. Increasing nitrogen rates consistently raised TA levels, particularly from additions pre-bloom, however the impact on pH varied between trials. YAN levels were generally increased by nitrogen addition, except from low rates of pre-veraison nitrogen in 2005-06. Post veraison applications did not increase nitrogen in 2006-07.

Berry phenolic levels were either unaffected or decreased by nitrogen addition and this appeared to have some interaction with exposure.

Nitrogen application rates that are excessive may lead to higher TA and decreased tannin, but are likely to also increase YAN. Botrytis levels may be impacted also. Nitrogen addition at 20 g N/vine, produced few changes in fruit quality, except for higher YAN

levels. Botrytis infection was increased over the control at this rate, but was less than the higher rate of addition. Conradie provides a nitrogen removal range of 1.39-1.93 kg N/ton of fruit. The historic yields from this block were around 8 tonnes per ha (P. Townsend, *pers. Comm.*), indicating a removal of 11.1-15.4 kg N/ha, or 7.5-10.4 g N/vine. Optimum addition rates can vary depending on soil organic matter (Löhnertz, 1991), soil moisture (Keller, 2004), vine rootstock (Holzapfel and Treeby, 2007) and canopy growth (Keller, 2004). Utilising a method of application that replicated fertigation, this trial found that 20 g N/vine was sufficient to increase YAN levels with minimal negative impact on the fruit.

Nitrogen timing in the first year of addition had a major impact on the fruit chemistry response, however made much less difference in the subsequent season. Post bloom applications appeared to delay fruit maturation. Care should be taken when deciding on the timing of addition to avoid high nitrogen availability during this period of the season.

4.4 Nitrogen addition to deficient grapevines (*Vitis vinifera* L.) cv Pinot Noir at different application timing and rates: Effects on fermentation dynamics and wine attributes

4.4a Introduction

Nitrogen availability can impact a number of factors that will influence wine quality. Increasing nitrogen availability to a vine that is deficient can increase canopy growth and improve the health of the vine leaves (Bell and Robson, 1999, Candolfi-Vasconcelos et al., 1997, Keller, 2004, Shawky et al., 2004, Winkler, 1970), which will increase the level of shading of the fruit (Smart, 1985, Smart, 1988) and also alter the photosynthetic capacity of the leaf (Candolfi-Vasconcelos et al., 1997). Nitrogen concentration within the vine will have a large influence on the nitrogen concentration of the fruit (Bath et al., 1991, Bettiga and West, 1991, Juhasz et al., 1984, Kliewer and Cook, 1974). Nitrogen levels in juice and must will have an influence during fermentation (Alexandre and Charpentier, 1998, Bell et al., 1979, Cramer et al., 2002).

Increasing canopy growth can reduce light reaching the fruit (Smart, 1985). This can lead to reduced production of phenolic compounds (produced in response to UV light (Cortell and Kennedy, 2006, Keller et al., 2003, Spayd et al., 2002)) and anthocyanins (stimulated by moderate warmth (Spayd et al., 2002, Bergqvist et al., 2001, Deis et al., 2009, Ristic et al., 2007)). Nitrogen may further reduce phenolic compound production directly (Keller and Hradzina, 1998, Pirie and Mullins, 1976). Major phenolics in wine include flavan-3-ols and tannins, and are important to wine quality, being a major component determining wine astringency (Gawel, 1998). They can also impact wine colour by stabilising anthocyanins red wines (Fulcrand et al., 2006, Somers and Evans, 1977). Anthocyanins are the basis of the red colour in red wine, and more anthocyanins can lead to a greater colour density, which can have a positive effect on wine preferences by tasters (Jackson et al., 1978).

Yeast assimilable nitrogen (YAN) consists of ammonium and free amino acids, and these will rise with increasing nitrogen availability. YAN deficiencies in musts and juice can lead to stuck or sluggish fermentations (Monteiro and Bisson, 1991, Henschke and Jiranek, 1993, Alexandre and Charpentier, 1998, Julien et al., 2000, Cramer et al., 2002) and the production of hydrogen sulphide (Henschke and Jiranek, 1993, Jiranek et al., 1995b, Hallinan et al., 1999, Gardner et al., 2002). This deficient level will be modified by yeast strain (Jiranek et al., 1995b, Gardner et al., 2002, Bell and Henschke, 2005).

Nitrogen may also impact on wine quality by influencing the level of a range of odour-active compounds. These include esters, several of which are derived from the metabolism of amino acids (Bell and Henschke, 2005, Guitart et al., 1999), and higher alcohols (Beltran et al., 2005, Gallander et al., 1990, Giorgessi et al., 2001, Webster et al., 1993). Esters contribute a range of aromas to the wine, and increases in vineyard nitrogen will increase the esters in wine (Ough and Lee, 1981, Webster et al., 1993, Giorgessi et al., 2001, Beltran et al., 2005, Linsenmeier et al., 2005, Miller et al., 2007a, Bell and Henschke, 2005). Higher alcohols at low rates can have a positive impact on wine quality, but a negative impact as levels increase. Higher alcohol production will initially be decreased as YAN is increased in a deficient ferment medium, but will increase again as YAN levels increase above moderate levels (Bell and Henschke, 2005).

While higher wine phenolic and anthocyanin concentrations that may come from reduced canopy growth as a result of limiting nitrogen are generally regarded as improving wine quality, stressed fermentations will have a negative impact. Nitrogen optimisation is important to maximise wine quality.

Previous chapters have determined that nitrogen addition to a block of Pinot Noir vines leads to increased vine vegetative growth, delay leaf senescence and increase leaf chlorophyll content, although were moderated in some cases by nitrogen rate or the timing of addition (see sections 4.2 p. 91 and 4.3 p. 121).

The trials outlined in the following chapter investigated the impact of nitrogen fertilisation of Pinot Noir vines on fermentation dynamics and wine quality parameters. A range of application dates and rates were compared. Particular attention was paid to the wine phenolic and anthocyanin content, and fermentation rates. The results were then compared to measurements taken in the field of vine canopy attributes. The main aim of these studies was to complete the field investigations on nitrogen application, and to increase the understanding of its impact on Pinot Noir wines in a cool climate region

4.4b Materials and Methods

Trials

Trials in this chapter include the nitrogen application and irrigation trial conducted in 2005-06, the nitrogen rate by timing trial, conducted in 2006-07 and 2007-08, and the nitrogen by exposure trial.

The nitrogen application and irrigation trial consisted of three rates of nitrogen (0, 17 and 51 g N/vine) and three irrigation rates (nil, standard and high). Treatments were applied at veraison in the 2005-06 season. Full trial layout is described in the materials and methods section (p. 22).

The nitrogen rate by timing trial consisted in 2006-07 of three nitrogen rate (20, 35 and 50 g N/vine) applied at four dates (pre-bloom, post bloom, pre-veraison and post veraison), and an untreated control. In 2007-08 nitrogen was reapplied to all treatments except the 35 g N/vine treatment, which was also excluded from monitoring. Full trial layout is described in the materials and methods section (p. 22).

The nitrogen by exposure trial involved an application of nitrogen at a rate of 100 g N/vine pre-bloom in the 2006-07 season. Fruit was then segregated into exposed fruit and shaded fruit, based on the location of bunches in the canopy. Full trial layout is described in the materials and methods section (p.23).

Materials and methods used in this trial are outlined in Table 4.4-1.

*Table 4.4-1 Analysis of trials investigating the impact of nitrogen on fermentation dynamics and wine attributes. Dates are given as month/year. Reference page lists the page in the general materials and methods for more detail. * - more information on this analysis below the table.*

Trial	*Analysis*	*Date*	*Reference page*
Nitrogen by irrigation trial	Winemaking and fermentation rate*	04/2006 04/2007	35, 36
	Somers analysis*	06/2007 (2007 wines)	37
Nitrogen timing by rate trial	Winemaking and fermentation rate*	04/2007 04/2008	36
	Somers analysis	08/2007 (2007 wines) 06/2008 (2008 wines)	37
	Tannin analysis	06/2008 (2008 wines)	37
	MS-e_nose	07/2008 (2008 wines)	38
Nitrogen by exposure trial	Winemaking*	04/2007	36
	Somers analysis	06/2008	37
	Tannin	06/2008	37

Winemaking and fermentation rate

Wines made in the nitrogen by irrigation trial in 2006 and 2007 were made using a punch-down cap management system.

Wines made in the nitrogen timing by rate trial were made using the punch-down cap management system, and in 2008 using the submerged cap management system.

Wines made in the nitrogen by exposure trial were made using the submerged cap system.

Wine colour measurement and Somers analysis

Wines were analysed by the modified Somers analysis developed by the Australian Wine Research institute, detailed in the materials and methods chapter.

In 2006, wines were assessed for colour density and hue, buffered for pH changes. These wines were re-measured using the full Somers analysis in 2008.

2007 wines were assessed when they were 3 months old, as were wines made in 2008. Wines made from the nitrogen timing by rate trial in 2007 were not assessed for tannin concentration, since the method had not been developed at that stage.

4.4c Results

Fermentation rates

Assessments of the drop in Baumé levels over the middle 48 hours of fermentation correlated to YAN in all years of the trial (Figure 4.4-1).

As a result of this correlation, treatment differences in fermentation rate were similar to the treatment differences in YAN in the juice (Table 4.3-21, Figure 4.3-6, Figure 4.3-7). In the 2005-6, treatments receiving 17 g N/vine fermented slower than treatments receiving either no nitrogen or 51 g N per vine (Figure 4.4-2A), while removing irrigation after veraison increased ferment rate (Figure 4.4-2B). In 2006-07 there were few

treatment effects on fermentation rate, although a rate effect did become apparent late in the ferment (Figure 4.4-2C). Rate of application had a clear impact of increasing fermentation rate in 2007-08 (Figure 4.4-2E). Timing of application did not produce major changes in ferment rate in either 2006-07 (Figure 4.4-2D) or 2007-08 (Figure 4.4-2F).

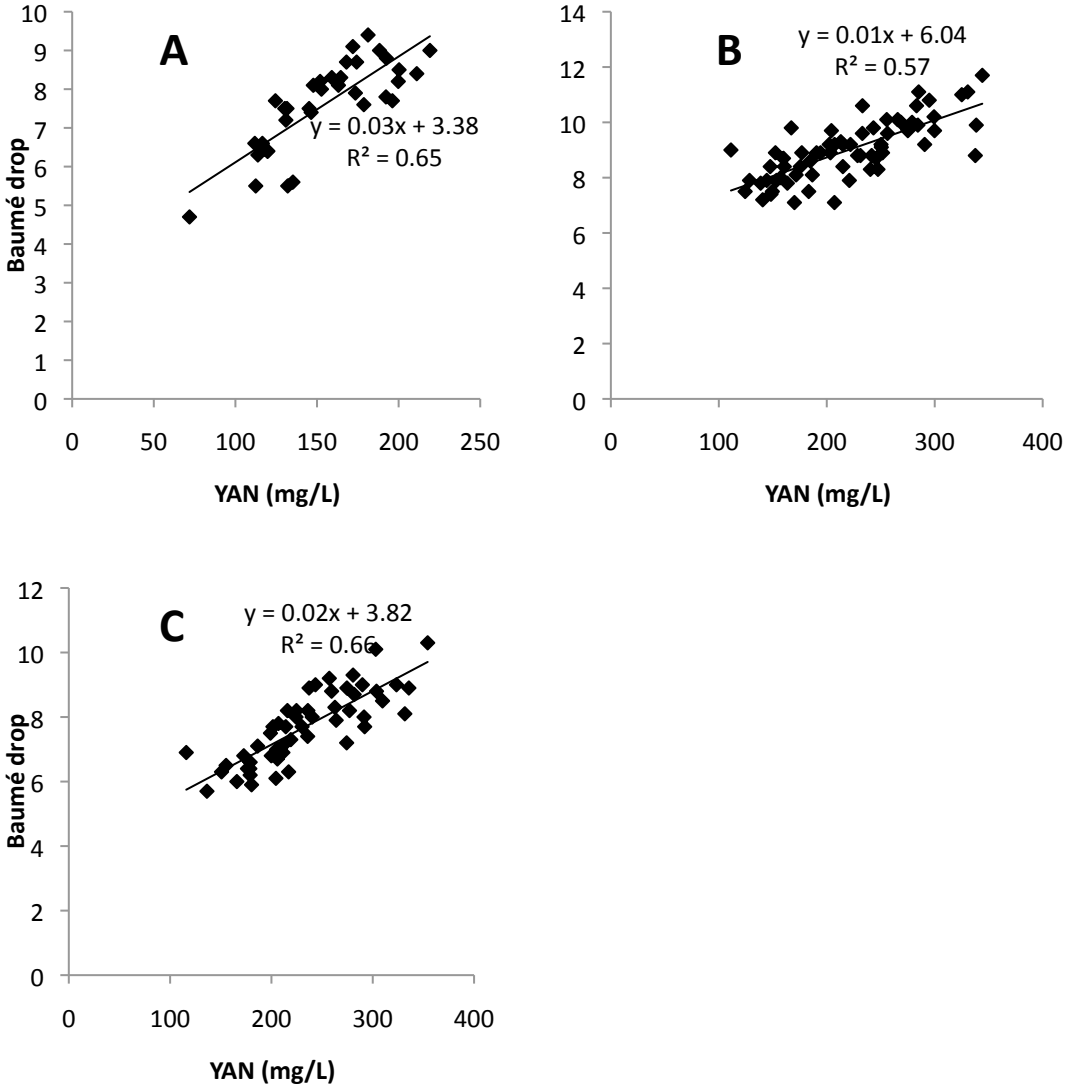

Figure 4.4-1 Baumé drop in the middle 48 hours of fermentation in response to YAN in the must in three years. A – Nitrogen by irrigation trial, 2005-06; B – Nitrogen rate by application timing trial 2006-07; C - Nitrogen rate by application timing trial 2007-08.

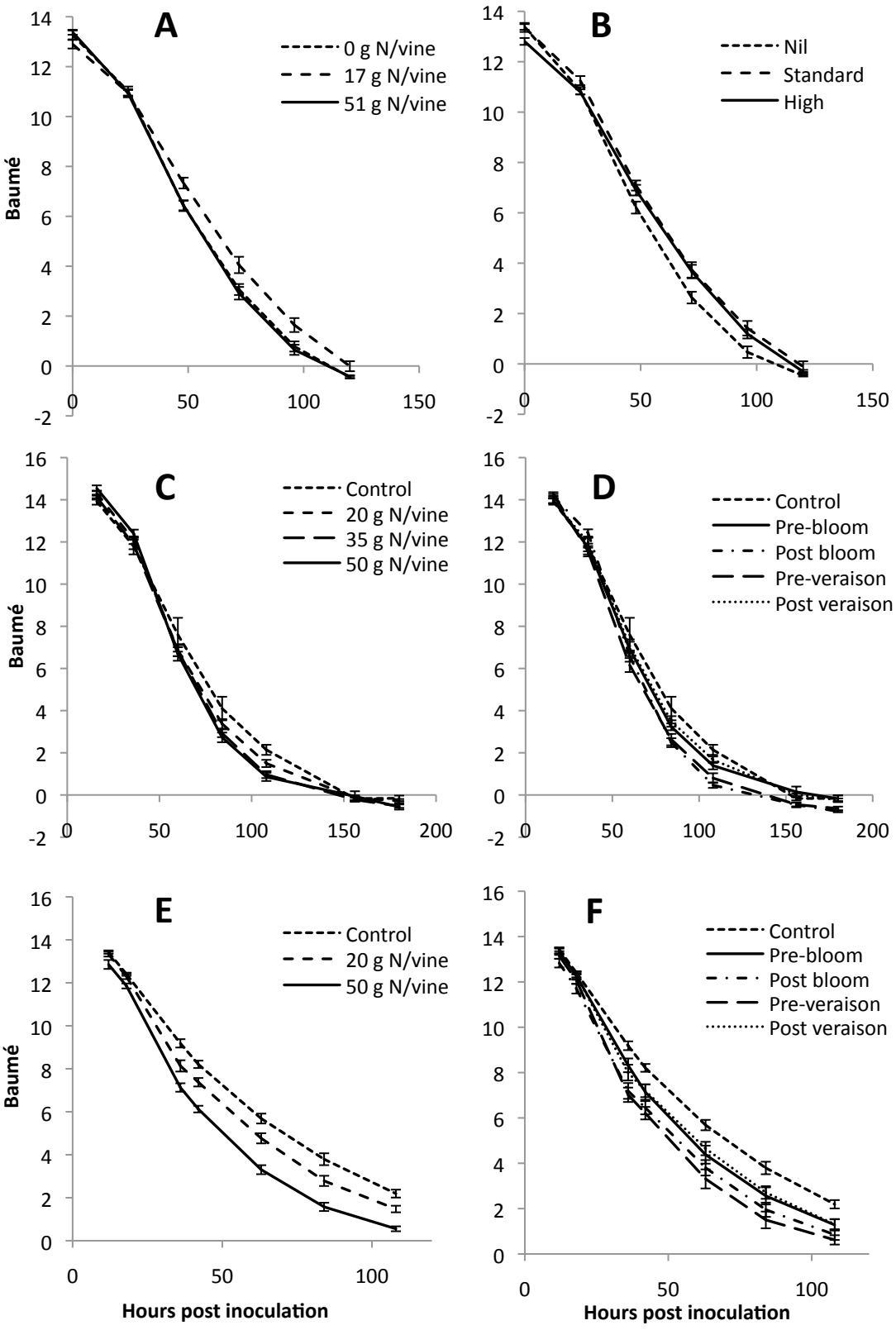

Figure 4.4-2 Fermentation curves in response to nitrogen and irrigation treatments. A – 2005-06 ferment response to nitrogen rate; B – 2005-06 ferment response to irrigation rate; C – 2006-07 ferment response to nitrogen rate; D – 2006-07 ferment response to timing of application; E – 2007-08 ferment response to nitrogen rate; F – 2007-08 ferment response to timing of application. Error bars represent standard error of the mean.

Wine chemistry – Somers analysis and tannin analysis

Somers analysis of wines made in 2005-06 did not have any significant differences in response to nitrogen fertilisation.

Somers analysis of wines made in the 2006-07 season following fertilisation of vines in the previous season in the nitrogen by irrigation trial indicated a nitrogen addition led to an increase in anthocyanin concentration (Table 4.4-2). There was also a non-significant trend towards increasing colour density (SO_2 corrected) as nitrogen rate increased (P = 0.057).

*Table 4.4-2 Somers analysis of wines made in 2006-07 from the nitrogen by irrigation trial. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Nitrogen Rate (g N/vine)	*Chem age 1*	*Chem age 2*	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*Colour density*	*Colour density (SO_2 corrected)*	*Hue*	*SO_2 resistant pigments*	*Somers phenolics (AU)*
0	0.27b	0.078	173a	14.4	3.6	4.9	0.77	0.80	28.6
17	0.26ab	0.076	176ab	14.6	3.7	5.1	0.77	0.82	29.3
51	0.25a	0.072	199b	13.9	3.9	5.4	0.76	0.82	29.6
Sig	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Juice analysis had identified an increase in total soluble solids (TSS) at harvest in 2006-07 from increased nitrogen rates applied at veraison the previous season (Table 4.3-13). Regression analysis examining correlations between wine colour density and total soluble solids (TSS) indicated that a difference in maturity of the fruit was linked wine colour density (Table 4.4-3).

*Table 4.4-3 Correlations between fruit maturity and wine colour in nitrogen by irrigation trial in 2006-07. R^2 values are above the diagonal, and correlation coefficient values are below. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	TSS	*Colour density (SO_2 corrected)*	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*SO_2 resistant pigments*	*Somers Phenolics (AU)*
TSS	-	0.54 (***)	0.34 (*)	n.s.	0.21 (*)	0.35 (**)
Colour density (SO_2 corrected)	0.74	-	0.70 (***)	n.s.	0.53 (***)	0.70 (***)
Total Anthocyanin (mg/L)	0.58	0.83	-	n.s.	n.s.	0.44(***)
Anthocyanin ionization %	n.s.	n.s.	n.s.	-	0.19 (*)	n.s.
SO_2 resistant pigments	0.46	0.73	0.38	0.44	-	0.42 (***)
Somers Phenolics (AU)	0.59	0.84	0.66	n.s.	0.65	-

Somers analysis of wines from the 06-07 season showed significant increases in the pre bloom nitrogen applications of anthocyanin ionization, colour density (natural and SO_2 corrected), and chemical age 2.

Table 4.4-4 Somers analysis of wines made in 2006-07 from vines treated with nitrogen at three different rates, applied at 4 timings during the season. PrB = pre-bloom; PoB = post bloom; PrV = pre-veraison; PoV = post veraison (P=0.05-0.01; ** P = 0.01-0.001; *** P <0.001; n.s. = no significant differences)*

Treatment	Rate	Chem age 1	Chem age 2	Total Anthocyanin (mg/L)	Anthocyanin ionization %	Colour density	Colour density (SO_2 corrected)	Hue	SO_2 resistant pigments	Somers phenolics (AU)
Control	0	0.26	0.073	199	16.8	4.35	5.23	0.74	0.83	32.4
Nitrogen	20	0.269	0.078	192	17.7	4.46	5.31	0.74	0.86	31.3
rate	35	0.276	0.079	179	18.0	4.25	4.99	0.75	0.82	29.7
(g N/vine)	50	0.290	0.083	185	18.1	4.49	5.16	0.75	0.89	30.3
	Sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Application	PrB	0.30	0.090b	193	19.7b	5.1b	5.8b	0.74	0.78	32.2
timing	PoB	0.27	0.078a	177	17.9a	4.2a	4.9a	0.76	0.96	28.9
	PrV	0.27	0.075a	184	17.2a	4.1a	4.9a	0.74	0.89	30.6
	PoV	0.27	0.077a	188	17.1a	4.2a	5.1a	0.74	0.81	30.1
	Sig	n.s.	*	n.s.	***	***	***	n.s.	n.s.	n.s.

The results in 2006-07 showed a highly significant correlation between SO_2-corrected colour density and total soluble solids, total anthocyanin concentration and degree of ionization, SO2-resistant pigments and phenolics (Table 4.4-5). There were also a significant correlation to between total soluble solids and anthocyanin ionization, SO_2-resistant pigments and phenolics. The strongest correlation to colour density was with wine phenolic concentration and with SO_2-resistant pigments.

Table 4.4-5 Correlations between wine colour density (SO_2 corrected), soluble solids, anthocyanin concentration and ionization, SO_2-resistant pigments and phenolics ,in 2006-07 in the nitrogen timing by rate trial. R^2 values in top right section of table, Pearson's correlation coefficients in the lower left section. (P=0.05-0.01; ** P = 0.01-0.001; *** P <0.001; n.s. = no significant differences)*

	TSS	Colour density (SO_2 corrected)	Total Anthocyanin (mg/L)	Anthocyanin ionization %	SO_2 resistant pigments	Somers Phenolics (AU)
TSS	-	0.24 (***)	n.s.	0.19 (***)	0.32 (***)	0.06 (*)
Colour density (SO_2 corrected)	0.49	-	0.49 (***)	0.19 (***)	0.75 (***)	0.65 (***)
Total Anthocyanin (mg/L)	n.s.	0.70	-	n.s.	0.13 (**)	0.42 (***)
Anthocyanin ionization %	0.44	0.44	n.s.	-	0.34 (***)	n.s.
SO_2 resistant pigments	0.57	0.87	0.36	0.58	-	0.52 (***)
Somers Phenolics (AU)	0.25	0.81	0.65	n.s.	0.72	-

In the second season of the rate by date trial (2007-08), Somers analysis showed very little difference in any treatment on any of the parameters. There was an increase in chemical age 2 as a result of fertilisation, and a decrease in hue value following pre-

bloom application of nitrogen (Table 4.4-6). SO_2-resistant pigments were increased in wines from vines that received nitrogen prior to veraison, compared to post bloom applications and wine from the control vines.

Table 4.4-6 Impact of nitrogen application date on Somers parameters in 2007-08. (P=0.05-0.01; ** P = 0.01-0.001; *** P <0.001; n.s. = no significant differences)*

N date	*Chem age 1*	*Chem age 2*	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*Colour density*	*Colour density (SO_2 corrected)*	*Hue*	*SO_2 resistant pigments*	*Somers phenolics (AU)*
Control	0.22	0.056a	223.1	16.2	4.2	5.1	0.68b	0.69a	33.1
PrB	0.22	0.059b	228.7	17.2	4.5	5.3	0.65a	0.74ab	31.8
PoB	0.23	0.060b	212.0	16.9	4.2	4.9	0.67ab	0.71a	29.8
PrV	0.23	0.061b	229.7	16.5	4.4	5.3	0.67ab	0.77b	32.2
PoV	0.23	0.060b	222.0	16.3	4.3	5.2	0.68b	0.74ab	31.4
Sig	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	*	n.s.

*Table 4.4-7 Correlations between colour density, anthocyanin concentration and ionization, SO_2 resistant pigments, phenolics and tannins in wines from the nitrogen timing by rate trial made in 2007-08. Top right section = R^2 values; bottom left section Pearson's correlation coefficients. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Colour density (SO_2 corrected)	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*SO_2 resistant pigments*	*Somers Phenolics (AU)*	*Tannin (g/L)*
Colour density (SO_2 corrected)	-	0.81 (***)	n.s.	0.70 (***)	0.64 (***)	0.26 (***)
Total Anthocyanin (mg/L)	0.90	-	n.s.	0.37 (***)	0.50 (***)	0.16 (**)
Anthocyanin ionization %	n.s.	n.s.	-	n.s.	n.s.	n.s.
SO_2 resistant pigments	0.83	0.61	n.s.	-	0.37 (***)	n.s.
Somers Phenolics (AU)	0.80	0.71	n.s.	0.56	-	0.81 (***)
Tannin (g/L)	0.39	0.90	n.s.	n.s.	0.56	-

Anthocyanin concentrations of wines made in the 2007-08 season were correlated to the anthocyanin concentration in the berry extracts, on a mg/g basis (R^2 = 0.60). Tannin in the wine was not correlated to the berry extract tannin concentration. Tannins decreased significantly in wine as a result of high rates of nitrogen fertilisation in 2007-08 (Figure 4.4-3). The 20 g treatment was not significantly different to the control.

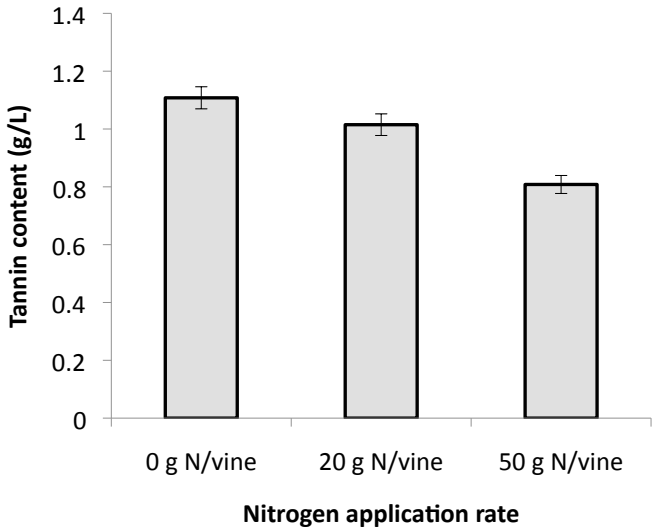

Figure 4.4-3 Tannin concentration in wine in response to nitrogen application rate in 2007-08. Error bars represent standard error of the mean.

Somers analysis of the wines from the trial comparing nitrogen level and exposure level found a number of significant differences. External bunches had more anthocyanin, phenolics, SO_2 resistant pigments and total pigments than internal bunches (Table 4.4-8). There was a significant interaction between the two factors for the Somers phenolics assessment (P=0.023), with unfertilised, exposed bunches having a significantly higher reading than all other treatment combinations. These changes were associated with higher colour density in wines made from external bunches. The wine tannin concentrations did not differ significantly as a result of bunch exposure (Figure 4.4-4).

Nitrogen addition resulted in less tannins in the wine (Figure 4.4-4). Hue was higher in wines from vines fertilised with nitrogen, and SO_2 resistant pigments were lower (Table 4.4-8).

Wine colour was significantly correlated to SO_2 resistant pigments, and also to wine tannins, however there was no correlation with wine anthocyanin concentration (Table 4.4-9).

There were no significant correlations between either berry extract anthocyanin concentration and wine anthocyanin concentration, or berry extract tannin and wine tannin concentration (Table 4.4-9).

*Table 4.4-8 Somers analysis of wines comparing the impact of nitrogen fertilisation of vines with bunch exposure in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Rate*	*Chem age 1*	*Chem age 2*	*Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*Colour density*	*Colour density (SO_2 corrected)*	*Hue*	*SO_2 resistant pigments*	*Total Pigments*	*Phenolics (AU)*
Position	Internal	0.39	0.15	93a	28	4.1	4.4	0.94	0.90	6.2	28.0
	External	0.38	0.14	114b	25	4.6	4.9	0.91	1.01	7.4	30.8
	Sig	n.s.	n.s.	*	n.s.	*	*	n.s.	n.s.	**	**
Nitrogen	0	0.40	0.16	105	27	4.5	4.8	0.88	1.05	7.0	31.4
(g	100	0.37	0.13	102	26	4.2	4.5	0.97	0.87	6.6	27.4
N/vine)	Sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	***

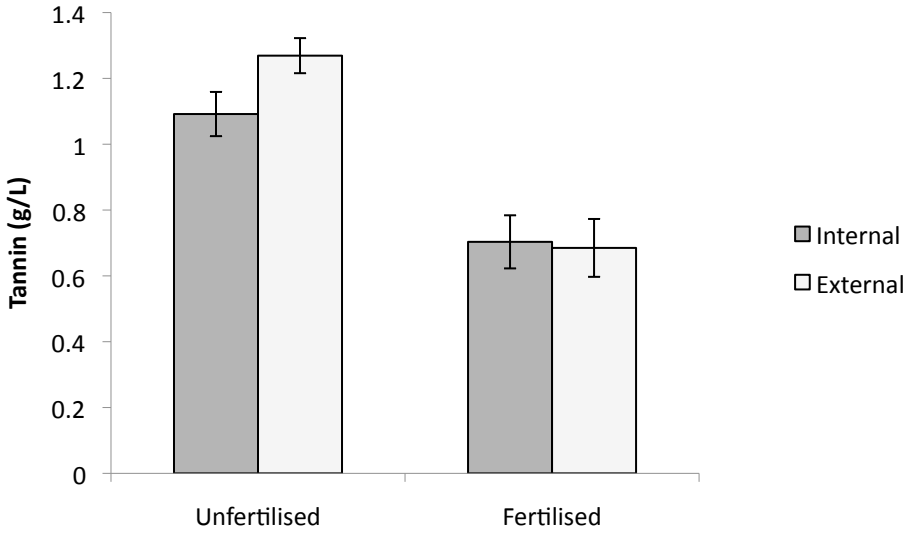

Figure 4.4-4 Wine tannin concentration as a result of separating shaded and exposed fruit on vines either fertilised with 100g N/vine or unfertilised in 2006-07. Error bars represent standard error of the mean.

*Table 4.4-9 Correlations between wine colour and other parameters in the nitrogen addition by exposure trial 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	Colour density (SO_2 corrected)	**Anthocyanin (mg/L)**	**Anthocyanin ionization**	**SO_2-resistant pigments**	**Total Phenolics**	**Tannin (g/L)**
Colour density (SO_2 corrected)	-	n.s.	n.s.	0.75 (***)	0.35 (***)	0.23 (*)
Anthocyanin (mg/L)	n.s.	-	n.s.	n.s.	0.27 (*)	n.s.
Anthocyanin ionization	n.s.	n.s.	-	0.19 (*)	n.s.	n.s.
SO_2-resistant pigments	0.86	n.s.	0.44	-	0.42 (***)	0.32 (**)
Total phenolics	0.84	0.52	n.s.	0.65	-	0.79 (***)
Tannin (g/L)	0.48	n.s.	n.s.	0.56	0.89	-

Principal component analysis (PCA) of MS e_nose analysis of the wines made in 2007-08 did not find any major groupings as a result of timing of nitrogen application. The first principal component, that accounted for 69% of the variation, showed some differentiation based on application rate (Figure 4.4-5), but without sensory data it was difficult to determine if this related to wine quality.

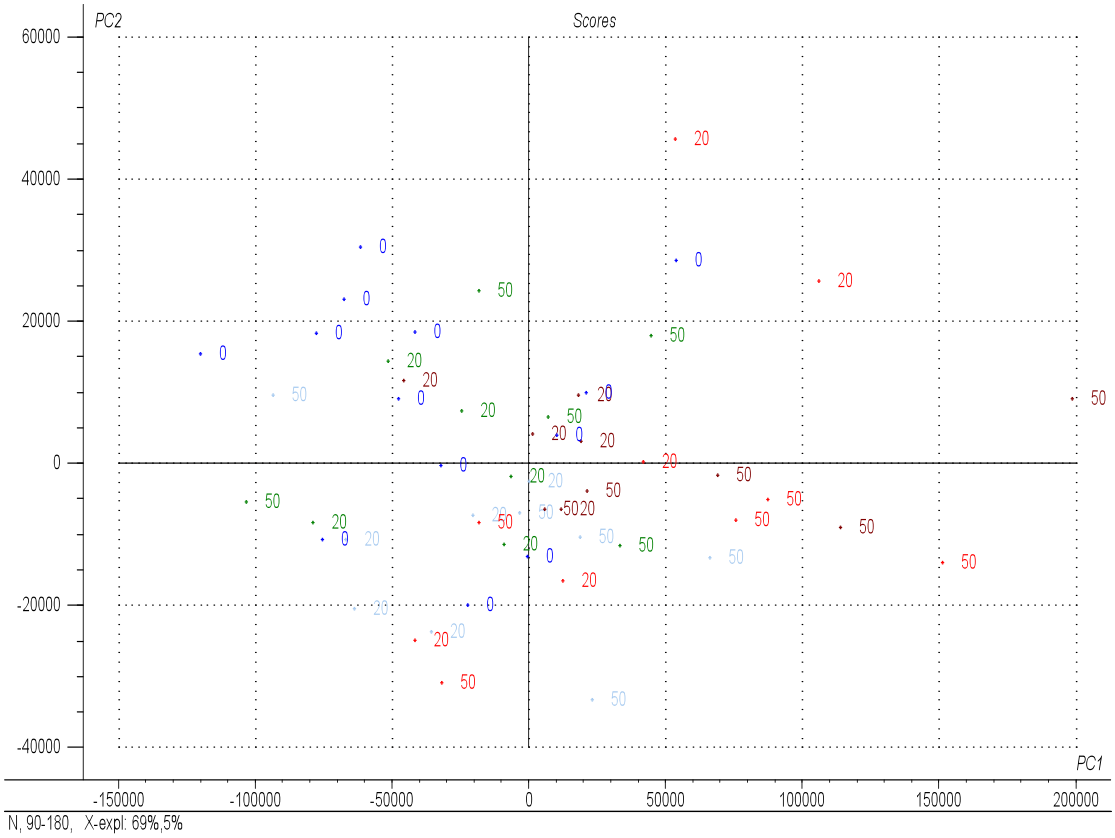

Figure 4.4-6 Principle component analysis plot from MS e_nose data of nitrogen trial wines in 2007-08. Blue values are controls; light blue values are pre-bloom applications; red values are post-bloom applications; red-brown values are pre-veraison applications; and green values are post-veraison applications. Numbers represent nitrogen rates.

4.4d Discussion

Fermentation rate and YAN

Temperature, starting sugar level, nitrogen level and yeast biomass are the major factors influencing fermentation rate (Coleman et al., 2007, Cramer et al., 2002, Varela et al., 2004). The small-scale fermentations used in the current study allowed temperature to be kept consistent between ferments by adjusting ambient air temperature, must sugar levels varied only in 2007 fruit that received early nitrogen, and yeast additions were consistent between ferments. With these factors kept constant, YAN levels were the major variable in fermentation rate, and strong correlations were seen between YAN and the Baume drop in the middle of the fermentation (Figure 4.4-1). YAN levels have been widely researched, and their importance to fermentation rates is well documented (Bell et al., 1979, Bell and Henschke, 2005, Cramer et al., 2002, Henschke and Jiranek, 1993). YAN impacts fermentation in two ways, increasing yeast biomass, and increasing the ability of yeast to conduct metabolism after the initial growth phase of fermentation. Higher YAN levels in the must at inoculation, particularly ammonium levels, will lead to greater yeast biomass (Beltran et al., 2005, Bell and Henschke, 2005). Fermentations that are too deficient in YAN are at risk of becoming stuck or sluggish (Alexandre and Charpentier, 1998). A minimal threshold has been proposed of a YAN level of 140 mg/L to ensure that fermentation does not cease as a result of nitrogen stress, although yeast vary in their nitrogen needs (Gardner et al., 2002), and in the case of red musts there will be a significant amount of nitrogen available to yeast from grape skins and seeds that will not

be reflected in juice YAN assessment (Stines et al., 2000). The lowest YAN concentration of musts in the present study was 135 mg/L, but no treatments were observed to not complete fermentation. There are several published examples of fermentations successfully finishing below 140 mg/L of YAN (Ugliano et al., 2008). Yeast will respond to further increases in YAN, and biomass increases have been reported with YAN levels up to 500 mg/L (Bisson, 1991), and optimum levels of up to 400-500 mg/L have been suggested (Henschke and Jiranek, 1993). Ferment rates responded to YAN increases in the present study across the whole range of YAN's measured, the highest of which was 259 mg/L.

Increasing nitrogen availability early in the season had a larger impact on must YAN than additions later in the year. Treatments which led to the largest increases in YAN included the pre-bloom and post bloom additions in the first year of the timing by rate trial (Figure 4.3-6), and any application date in the second year of this trial (Figure 4.3-7), when nitrogen was carried over from the previous season (Table 4.2-10). Treatments where nitrogen was added around or after veraison had much less impact on YAN (Figure 4.3-6), and may even lead to a reduction (Table 4.3-21). Therefore, these trials demonstrate that fermentation rate is increased more by fertiliser additions aimed to increase early nitrogen availability. Vineyard nitrogen addition leading to increased YAN levels have been demonstrated to increase fermentation rate (Spayd et al., 1995), although the impact of timing itself has not been investigated. The research here indicates that the timing of nitrogen addition will alter its impact on YAN and fermentation rate. The potential for nitrogen addition to decrease YAN, in particular, may alter optimum nitrogen application date.

Wine colour density, anthocyanins and phenolics

Nitrogen additions pre-bloom in 2006-07 led to an increase in wine colour density (Table 4.4-4). The positive correlation between wine colour density and soluble solids in both this trial (Table 4.4-5) and also in the 2006-07 fruit from vines that had received nitrogen at veraison in the previous year (Table 4.4-3) indicates that the colour density increase was associated with increased grape maturity. Fruit maturity was increased following pre-bloom nitrogen additions in 2006-07 (Table 4.3-16) and veraison nitrogen addition the previous season. Colour density increases as a result of nitrogen addition at veraison the preceding season were almost significant (P=0.057). Pre-bloom nitrogen addition in 2006-07 also increased level of veraison development (Figure 4.3-3), implying that these grapes had a longer period in the second phase of ripening (Coombe and McCarthy, 2000), since they were harvested on the same day.

Wines made in 2006-07 from vines that received nitrogen prior to bloom in the same season had an increased percentage of anthocyanin ionization (Table 4.4-4). Anthocyanin ionization in 2006-07 in the timing by rate trial was correlated with colour density. Anthocyanin ionization can be altered by wine pH or SO_2 concentration (Jackson, 1994, Somers and Evans, 1977), copigmentation with other pigments, or binding with other compounds to form new pigments (Romero-Cascales et al., 2005, Cheynier et al., 2006, Fulcrand et al., 2006). This binding is generally referred to as "polymerisation", although there are a number of potential compounds that are not polymers (Cheynier et al., 2006). Copigmentation and polymerisation reactions increase anthocyanin stability, and also increase the degree of anthocyanin ionization and hence pigmentation due to absorbance at visible wavelengths (Ribéreau-Gayon and Glories, 1986, Cheynier et al., 2006). Free SO_2 concentration and pH changes are unlikely to influence anthocyanin ionization in this trial, since there was no variation in juice pH between treatments (Table 4.3-16), SO_2 was added in uniform quantities to each wine (although free SO_2 concentration was not

assessed prior to these measurements), and test samples used buffered, SO_2-corrected ethanol solutions. Also the modified Somers analysis used pH buffered, ethanol solutions containing acetaldehyde to remove SO_2 effects. Dilution also reduces copigmentation effects; therefore an increase in incorporation of anthocyanin into other pigments is likely to be the cause of the increase in ionized anthocyanin (Somers and Evans, 1977, Ribéreau-Gayon et al., 2000, Cheynier et al., 2006, Fulcrand et al., 2006). These polymers and other associated compounds are included in the Somers measure of SO_2-resistant pigments. Wine phenolic concentration and SO_2-resistant pigments were positively correlated to colour density in all wines (Table 4.4-3, Table 4.4-5, Table 4.4-7, Table 4.4-9), indicating that the interactions that anthocyanins have with other compounds in the wine are more important to wine colour density than the anthocyanin concentration alone. It is noted that there were no significant differences between the phenolic content or SO_2 resistant pigments in this trial of different wines from 2006-07 (Table 4.4-4). The cause of the increased anthocyanin ionization in these wines therefore remains unexplained.

Phenolic concentration has been reported to decreases both with higher nitrogen availability (Keller and Hradzina, 1998, Pirie and Mullins, 1976) and more shading of the fruit (Cortell and Kennedy, 2006, Keller et al., 2003, Spayd et al., 2002). The impact of light was evident from the exposure by nitrogen trial, where exposed bunches in 2006-07 had a higher anthocyanin and phenolic concentration than bunches from the interior of the canopy (Table 4.4-8). A reduction in tannin after application of 50 g N/vine nitrogen was observed in the 2007-08 season (Figure 4.4-3), and following addition of 100 g N/vine in 2006-07 (Figure 4.4-4), however there was no significant change in wine phenolics from nitrogen addition in either trial. The decreases in tannin were only apparent at high rates of nitrogen application.

Wines made in 2006-07 from vines fertilised at veraison the previous season had increased total anthocyanin concentration (Table 4.4-2), which was correlated to the colour density, as well as to total soluble solids (Table 4.4-3). Anthocyanins become more extractable from the grape cells with higher fruit ripeness as a result of degradation of cell walls (Rolle et al., 2009), and anthocyanin concentration in the fruit increase with ripening (Rolle et al., 2009, Guidoni et al., 2008, Robinson and Davies, 2000). However, fruit anthocyanin was not measured in 2006-07, therefore it is not possible to determine whether changes were as a result of increased extraction or different grape anthocyanin concentrations. Other researchers have noted changes in fruit anthocyanin concentration in response to nitrogen addition. Some have observed that additional nitrogen has led to increases in anthocyanin concentration (Delgado et al., 2004, Ewart and Kliewer, 1977), while others have seen reductions (Keller et al., 1999, Hilbert et al., 2003, Wade et al., 2004) and still others have observed no change (Bell et al., 2009). Grape tissue cultures have shown that production of some anthocyanins may increase as ammonium availability rises while others can drop (Do and Cormier, 1991a). Increasing the sugar to nitrate ratio can stimulate anthocyanin production (Pirie and Mullins, 1976, Do and Cormier, 1991b).

The impact of exposure to sunlight on anthocyanin concentration in wines was apparent from the increase in anthocyanin concentration on exposed bunches compared to shaded bunches (Table 4.4-8) in 2006-07, however nitrogen did not alter wine anthocyanin concentration in any other trials apart from the increase observed in the 2006-07 season following nitrogen application at 51 g N/vine the preceding veraison (Table 4.4-2). It is possible that reports in the literature of a nitrogen impact on anthocyanin content may be due to changes in light exposure in response to increased canopy growth stimulated by nitrogen availability, and not nitrogen directly. Anthocyanins are known to increase in

response to exposure to light and in Cabernet Sauvignon grapes anthocyanin production is increased when berry temperature increases from 15°C to 25°C (Deis et al., 2009), while excessive temperatures may reduce anthocyanin production (Bergqvist et al., 2001, Spayd et al., 2002). The mean maximum temperatures recorded at the nearest Bureau of Meteorology station for February and March are 21.1°C and 19.9°C respectively, therefore increases in berry temperature above ambient temperature as a result of increased exposure may lead to increasing anthocyanin content in the fruit. Berry extract analysis did not indicate a significant increase in anthocyanin in response to exposure, and the two measures were not correlated.

MS e_nose

The results of the MS e_nose analysis did not indicate any distinct, systematic changes in wine volatile aroma in response to nitrogen treatment in the 2007-08 season (Figure 4.4-6), although there was some clustering based on nitrogen application rate. YAN concentration can have an impact on wine volatile compounds. Hydrogen sulphide is produced by yeasts when nitrogen availability is low (Jiranek et al., 1995b) and will contribute to unpleasant 'reduced' flavours in wine (Ribéreau-Gayon et al., 2000), although these flavours have a very low odor threshold so are unlikely to be detected by the MS e_nose. Esters are primary metabolites of amino acid metabolism by yeast (Henschke and Jiranek, 1993), and the amount of amino acid present can influence the ester concentration (Miller et al., 2007a). Further analysis of the impact of nitrogen on Pinot Noir wines is warranted, particularly considering recent advances in detection and importance of many potentially important volatile compounds (Fang and Qian, 2005, Fang and Qian, 2006).

Conclusion

Nitrogen fertilisation in the vineyard at both high and moderate application rates decreased fermentation times, due to a strong correlation between ferment rate and YAN. Fertiliser application may be able to reduce winery nitrogen supplementation.

Applications of nitrogen in some seasons may increase fruit maturity at harvest. In this trial increases in maturity were associated with increases in the colour density of the wine, which can have a bearing on the consumer perception of quality. This occurred in only one season, for reasons that are not known.

Moderate rates of nitrogen application were successfully able to increase fermentation rate, with no major impact on wine spectral characteristics. Higher rates of nitrogen, while decreasing ferment rates further still, did lead to a reduction in wine phenolics and tannins in some trials. Phenolic compounds were very important to wine colour density, and have other important organoleptic qualities, particularly to do with wine mouthfeel (Gawel, 1998).

This trial indicates that moderate applications of nitrogen can be made to Pinot Noir with little or no negative impact on wine quality, and potential benefits from improved ferment health. High rates should be avoided, with increased incidence of botrytis and decreases in phenolic content resulting.

4.5 Comparisons between the impact of supplementing must nitrogen by vine nitrogen applications or by winery applications on wine attributes

4.5a Introduction

The importance of nitrogen in winemaking has been the subject of significant research over the last thirty years. Fermentation dynamics will change under different levels of available nitrogen, and there can be changes in a number of compounds that contribute to the fermentation bouquet (Bell and Henschke, 2005).

Ensuring that there is adequate nitrogen for the requirements of yeast during fermentation can increase wine quality (Ough and Lee, 1981, Bell et al., 1979). Fermentations carried out under conditions of nitrogen deficit can lead to stuck or sluggish ferments (Alexandre and Charpentier, 1998, Coleman et al., 2007, Henschke and Jiranek, 1993, Monteiro and Bisson, 1991), and can also lead to the production of volatile sulphur compounds, in particular hydrogen sulphide. Stuck and sluggish ferments can be costly to manage and can lead to the growth of non-desirable microbes. Production of hydrogen sulphide can lead to unpleasant wine aromas, and can also be further reduced to other volatile compounds with undesirable aromas (Bell and Henschke, 2005).

Yeast are able to metabolise a range of nitrogen compounds, including proteins, amino acids, peptides and ammonium (Henschke and Jiranek, 1993), but the highest proportions of nitrogen come from amino acids and ammonium. In anaerobic respiration, only free alpha amino acids are metabolised, and this portion of the nitrogen is called free amino nitrogen (FAN). The total nitrogen available to yeast is referred to as yeast assimilable nitrogen (YAN) (Dukes and Butzke, 1998), being the sum of FAN and ammonium N. Researchers have suggested that a minimum YAN of 140 mg/L is required to ensure a ferment does not become stuck or sluggish (Bely et al., 1991), however this value should only be treated as a guideline, since different yeast strains have different nitrogen requirements. Also, YAN assessment of red must juice will not include the portion of nitrogen that is in the skins and seeds, however these tissues may contribute over 30% of the nitrogen available to fermentation (Stines et al., 2000). Nitrogen deficiency at the beginning of a fermentation will lead to a reduction in yeast biomass, which will decrease fermentation rates (Varela et al., 2004). At low to moderate YAN levels, yeast will respond to addition of nitrogen sources during fermentation by increasing the rate of fermentation and decreasing the time taken to consume all available sugars. This response has been observed with incremental YAN increases up to 500 mg/L (Bisson, 1991), much above the threshold level of 140 mg/L.

Increasing the nitrogen availability to vines can increase YAN levels in harvested fruit (Holzapfel and Treeby, 2007). YAN may also change in response to other factors. This allows YAN manipulation in the vineyard, which is one option for increasing must YAN. Another is the addition of diammonium phosphate (DAP) directly to musts in the winery.

Adjustment of YAN in the vineyard will alter more than just must nitrogen levels. Shoot growth may be stimulated, leaf health improved, disease levels may increase, anthocyanin concentration can change (Hilbert et al., 2003), and phenolic compounds may decrease (Delgado et al., 2004). These impacts may alter wine attributes separately to the impact of different YAN levels.

DAP nitrogen is different in composition to natural YAN nitrogen. YAN generally contains the majority of its nitrogen as amino acids, and the minority as ammonium, while DAP provides all its nitrogen as ammonium. The production of higher alcohols and esters can contribute to wine aroma and are influenced by the must YAN, in particular the composition and amount of the free amino acids (Rapp and Versini, 1991, Ough and Lee, 1981).

Ugliano et al. (1998) found that anthocyanin concentration in wines have also been observed to increase after addition of DAP, although the mechanism is not understood. These researchers go on to speculate that it may be a result of a longer exposure to higher alcohol wines or to increased cap temperatures as a result of more rapid fermentation, or that there may be an interaction with the yeast. This study also found that DAP addition increased acetate esters, but did not affect higher alcohol concentration.

The timing of DAP addition for optimum effectiveness can alter fermentation kinetics. When added during the yeast growth phase, DAP will stimulate increased cell division and lead to an increased yeast population, compared to later additions which will alter rate but not population (Beltran et al., 2005). However, the increased microbial activity that results from the yeast addition at this stage can create the risk of a ferment overheating, and the recommendations for the maximum benefit from DAP addition are to add it at one-third to half way through the ferment (Bell and Henschke, 2005).

This trial was established to examine the effect on fermentation dynamics and wine attributes of managing YAN levels by either adjusting YAN in the vineyard with nitrogen fertiliser addition to vines, or by addition of DAP directly to ferments.

The impact of timing of DAP additions were also investigated, to allow an increased understanding of how this impacts fermentation of Pinot Noir. Adding DAP at the start of fermentation replicates the nitrogen availability in the juice (although there will be some nitrogen released later from skins and seeds), however the ability to add the nitrogen at different stages of fermentation is one advantage of DAP over endogenous nitrogen, therefore it was included in the trial.

The aim of the trial was to generate information that can assist in decision making for improved nitrogen management, and also to provide additional understanding of nitrogen dynamics for discussion within the context of the author's PhD thesis, investigating the impact of leaf health on wine quality by manipulating nitrogen levels.

4.5b Materials and Methods

Vineyard layout

This trial involved applying fertiliser to a group of vines at times and rates designed to increase YAN but minimise vegetative growth, based on responses to nitrogen observed in previous trials. 40 g N/vine was added, split between post veraison and pre-bloom timing. A second, larger group of vines was left unfertilised. At harvest fruit from fertilised vines were bulked together, as was fruit from unfertilised vines. Trial layout is described in more detail in the general materials and methods section (p.23).

Point quadrat data, leaf chlorophyll data and pruning weights were collected to determine what differences were made on the vine canopies as a result of nitrogen fertilisation.

A summary of the methods used in this trial is given in Table 4.5-1. For further detail on layout and materials and methods, please refer to the general materials and methods

section (p. 23).

*Table 4.5-1 Methods summary for trials comparing field nitrogen to winery-applied nitrogen. Reference page directs to the full method description in the materials and methods section. TA – Titratable acidity; TSS – total soluble solids; YAN – yeast assimilable nitrogen. * - more information on this analysis below the table.*

Analysis	*Date*	*Reference page*
Leaf chlorophyll estimate	27/03/08	26
Point quadrat	13/03/08 25/03/08	26
Pruning weight assessment	4/07/08	28
Yield at harvest	31/03/08	34
Juice pH, TA and TSS	2/04/08	33
YAN*	2/04/08 15/04/08	34
Winemaking and fermentation rate*	04/08	36
Somers analysis	06/08	37
Tannin analysis	06/08	37
MS-e_nose	07/08	38
Sensory analysis*	15/10/08	

Winemaking

The total weight of fruit harvested was 67 kg for the nitrogen treated fruit, and 133 kg from unfertilised vines. Fruit was allocated to 20 L fermenters ensuring there was a similar amount in each vessel, of around 11 kg of must, with 12 fermenters with fruit from nitrogen treated vines and 5 fermenters with fruit from unfertilised vines. These were divided up as shown in Table 4.5-2. The ferments were managed as a submerged cap ferment.

Table 4.5-2 Treatments and number of fermenters used in each

Treatment	*Number of Fermenters*
Field Nitrogen	5
Full DAP addition to must prior to inoculation	3
Full DAP addition at 1/3 ferment	3
Split DAP addition (50% prior to inoculation, 50% at 1/3 ferment)	3
Control	3

YAN analysis

Initial analysis was on a subset of the juices.

Samples were then taken from all fermenters prior to inoculation but following DAP addition in the relevant treatments, and frozen for analysis at a later date.

Sensory analysis

A panel of four winemakers were used, and the tasting was structured based on advice from the Australian Wine Research Institute's sensory department (Francis, L., *pers.*

comm.). This involved a preliminary tasting of four wines expected to represent the range of the wines in the trial. In the preliminary tasting, participants were asked to note descriptors for each category of aroma, mouthfeel and finish, and then these descriptors were listed for all to see. Each wine and each individual descriptor was discussed, and a term of descriptors that all tasters agreed upon was created. The final descriptor list consisted of:

- Tannin grain
- Tannin quantity
- Floral
- Herbal
- Rhubarb
- Fruit ripeness
- Cherry
- Strawberry
- Plum
- Earth
- Forest floor
- Reduced
- Pepper
- Almond

A tasting sheet was then created with two sections. In one section, tasters were asked to rate wines on the 20-point scale in common use in Australian wine judging. In the other section, each descriptor was assessed, with tasters grading the quantity of each attribute by placing a mark to bisect a 100 mm solid, unmarked line. The distance from the beginning of each line to the mark was later measured and this was used as the value for statistical analysis.

Each bottle was randomly numbered. Wines were tasted in two repetitions, with a separate bottle used for each repetition. With the total number of treatments being 17 per repetition, three tasting flights were used, two with 6 and one with 5 wines. Wines were tasted in standard XL-5 tasting glasses, on a white, well-lit bench. Each taster started on a different repetition, so none tasted the same group of wines at any time. Tasters did not discuss wines while tasting.

Results were analysed using standard ANOVA analysis, with the taster included as a factor.

4.5c Results

Leaf health

Leaf chlorophyll was increased by the addition of nitrogen.

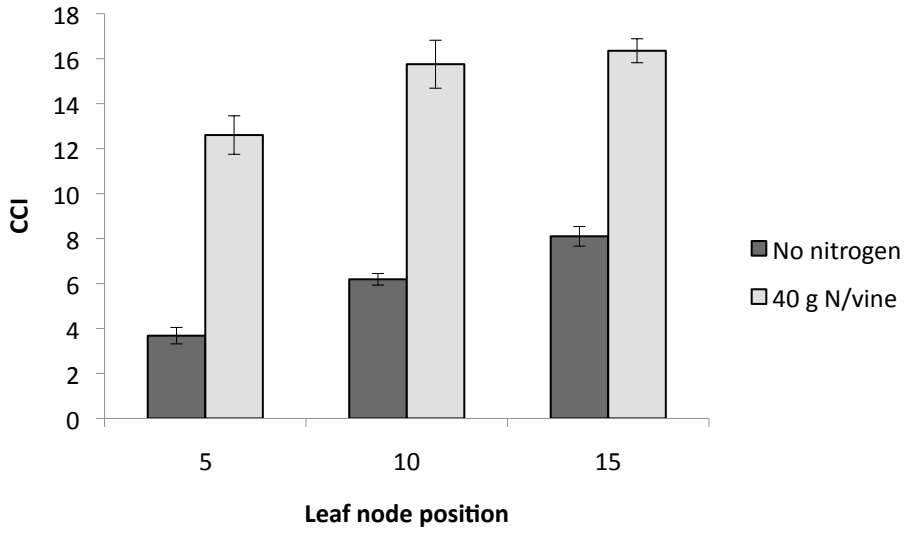

Figure 4.5-1 Chlorophyll concentration estimations (CCI) at different node positions on nitrogen fertilised and unfertilised vines, assessed on the 27th March 2008. Error bars represent standard error of the mean.

Point Quadrat Analysis

Point quadrat analysis at the 13th of March indicated that there was very little difference between treatments in terms of canopy density, with the only difference of significance being a decrease in the number of yellow leaves on vines that had received nitrogen applications (Table 4.5-3).

By the 25th of March the treatment differences were more apparent (Table 4.5-3), with nitrogen treated vines having more leaves per insertion. This can be seen in the higher leaf layer number, internal leaf counts and external leaf counts. The difference in yellow leaves was also still apparent.

*Table 4.5-3 Point quadrat analysis at two dates comparing nitrogen fertilised and non-nitrogen fertilised vines. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

	13th March			***25th March***		
	Unfertilised	Fertilised	Sig	Unfertilised	Fertilised	Sig
Leaf Layer Number	2.1	2.0	n.s.	1.5a	1.9b	*
Internal Leaves	0.5	0.4	n.s.	0.2a	0.4b	*
External Leaves	1.6	1.6	n.s.	1.3a	1.5b	*
Yellow Leaves	0.4b	0.1a	**	0.3b	0.1a	*

Pruning weight

Nitrogen addition in the field did not lead to any significant changes in shoot growth, as measured by pruning weight and mean cane weight analysis (Table 4.5-4).

*Table 4.5-4 Pruning weight analysis following split nitrogen addition in 2006-07. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Pruning weight (kg)*	*Mean Cane Weight (g)*
Field Nitrogen	1.41	53.8
No Field Nitrogen	1.40	51.2
Sig	n.s.	n.s.

Yield

Nitrogen addition did not lead to any significant differences in yield per vine nor in disease levels (Table 4.5-5).

*Table 4.5-5 Yield of each group of field treatments. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Yield per vine (kg)*	*Botrytis affected bunches per vine*
Field Nitrogen	3.28	4.4
No Field Nitrogen	3.50	3.2
Sig	n.s.	n.s.

Juice chemistry

There were no significant differences between TSS values, pH or TA (Table 4.5-6).

*Table 4.5-6 Juice chemistry at harvest. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Brix*	*pH*	*TA*
Field Nitrogen	24.1	3.5	5.9
No Field Nitrogen	24.0	3.5	5.9
Sig	n.s.	n.s.	n.s.

Yeast assimilable nitrogen rose as a result of nitrogen application in the field, with higher FAN and higher ammonium concentration. YAN also rose following DAP addition, as a result of increased ammonium nitrogen.

*Table 4.5-7 Free amino acid nitrogen (FAN), ammonium nitrogen and yeast assimilable nitrogen (YAN) concentration of musts following initial DAP addition but prior to inoculation. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*FAN (mg/L)*	*Ammonium N (mg/L)*	*YAN (mg/L)*
Field Nitrogen	238b	61b	298d
All DAP added to must	168a	78d	246c
All DAP added mid ferment	162a	35a	197a
Split DAP add	167a	65c	232b
Control	165a	35a	201a
Sig	***	***	***

Fermentation rate

Increased YAN levels led to an increased rate of fermentation. Field nitrogen treatments,

with the highest YAN level of all treatments, fermented significantly faster than all other treatments. Adding all the DAP prior to inoculation gave a significant boost to fermentation rate, however 12 hours after the mid-ferment nitrogen addition there was no difference in level of sugars consumed from the start of fermentation between any DAP addition timing treatments (Figure 4.5-2). The control vines were significantly slower than all those that received nitrogen, either in the field or as DAP.

Fermentation rates were monitored until the ferments were pressed. While they were not monitored after this date, subsequent testing determined that all ferments had proceeded to dryness.

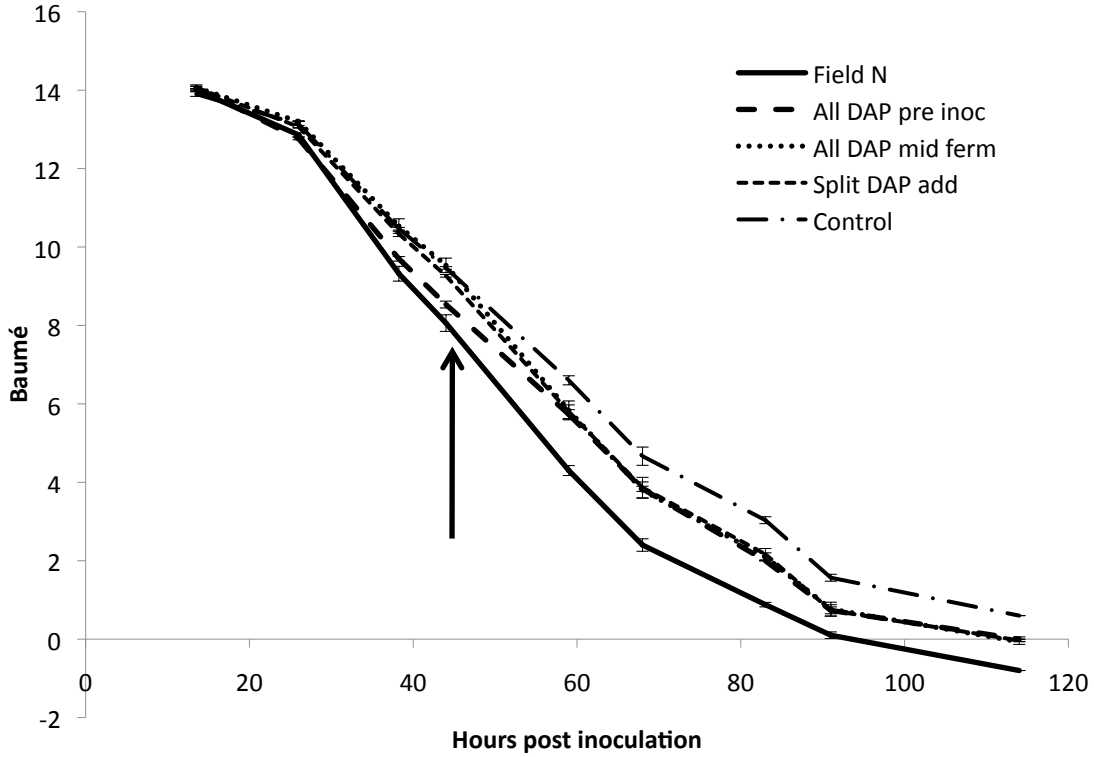

Figure 4.5-2 Fermentation progression curve. Vertical arrow represents the timing of the mid ferment DAP addition. Error bars represent standard error of the mean.

Somers analysis

There were a number of Somers characteristics that were altered by nitrogen source, and by timing of addition of DAP. Anthocyanin ionization was lowest in wines receiving nitrogen in the field, or those that received a split dose of DAP. Wines that received all nitrogen prior to inoculation, mid ferment and controls all had increased anthocyanin ionization levels (Table 4.5-8).

Colour density was increased in treatments that received an early addition of DAP. The highest SO_2-corrected colour density was in the wines where the full amount of DAP was added to the must before inoculation, which was significantly higher than all but the split DAP treatment, where half the total DAP addition was added prior to inoculation. Hue was significantly higher in the control than in all other treatments.

*Table 4.5-8 Somers analysis of wines made with nitrogen added in the field or at different timings in the winery. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Chem age 1*	*Chem age 2*	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*Colour density*	*Colour density SO2 corrected*	*Hue*	*Somers phenolics (AU)*
Field N	0.21b	0.047	252	14.8a	4.06a	4.84a	0.61a	29.7
DAP in must	0.18a	0.044	262	17.2c	4.60b	5.42b	0.60a	32.0
DAP mid ferment	0.19a	0.047	242	16.6c	4.22a	5.04a	0.61a	29.5
DAP split	0.20ab	0.049	249	15.3ab	4.15a	5.14ab	0.62a	30.4
Control	0.18a	0.048	229	16.4bc	4.03a	5.01a	0.64b	28.6
Sig	**	n.s.	n.s.	**	**	*	**	n.s.

There was no impact on wine phenolics levels as a result of field nitrogen addition, nor any of the winery treatments (Table 4.5-9).

*Table 4.5-9 Dambergs wine parameters of wines made with nitrogen added in the field or at different timings in the winery. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Tannin (Dambergs) (g/L)*	*Total pigments*
Field N	0.91	13.7
Dap in must	1.02	14.2
DAP mid ferment	0.91	13.1
DAP split	0.96	13.6
Control	0.89	12.4
Sig	n.s.	n.s.

Table 4.5-10 Regression analysis output between SO2 corrected colour density and other wine chemical attributes in the field versus winery nitrogen supplement trial in 2007-08.

	Tannin (Dambergs)	**Total phenolics (Somers)**	**Anthocyanin ionization**	**Total Anthocyanin**
R^2	0.76	0.65	0.22	0.30
Sig	***	***	n.s.	*

MS-e_nose analysis

Principal component analysis on MS e_nose output indicated that there were treatment clusters for all treatments, indicating that there were differences in the volatile components between treatments (Figure 4.5-3). Treatments 4 and 5 were clustered together.

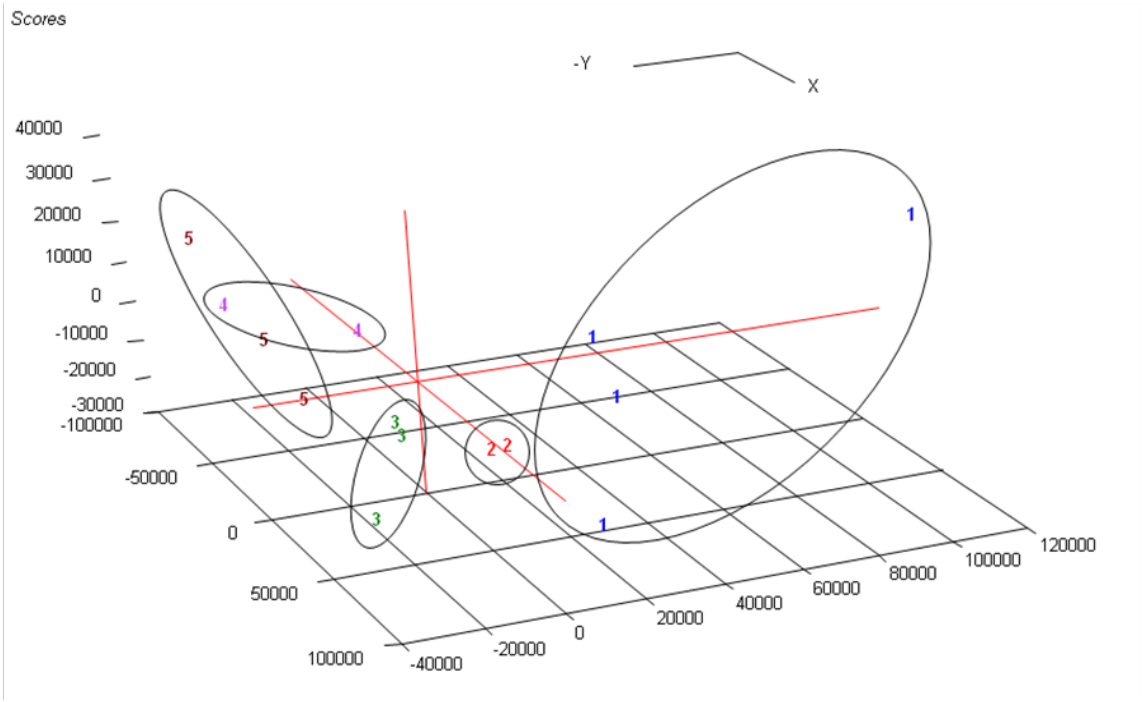

Figure 4.5-3 PCA output from MS e_nose analysis of wines in the DAP against field nitrogen trial (outlier samples removed), for the three main contributing variables. Trials are: 1 = Field nitrogen; 2 = DAP pre-inoculation; 3 = DAP at 48 hours; 4 = split DAP addition; and 5 = control

Sensory analysis

Tasting of the wines did not find many consistent differences between the descriptors that were rated by the tasters, however there were still organoleptic differences in "sweet spice" and "forest floor" characters (Figure 4.5-4). Sweet spice character was seen to be higher in wines from the control must (no field or winery N addition). Forest floor characteristics were lower in wines that received DAP, particularly when added to the must prior to inoculation.

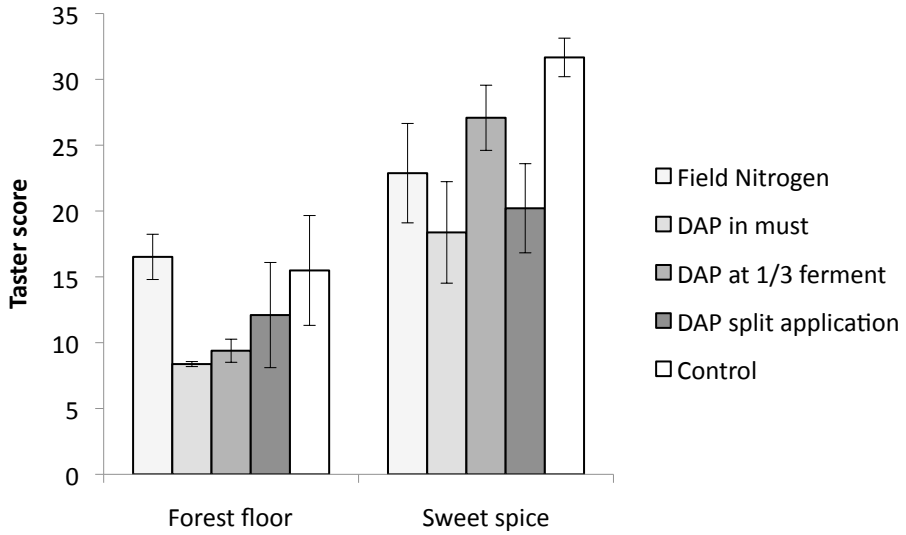

Figure 4.5-4 Average scores for forest floor and sweet spice in field nitrogen against DAP tasting. Error bars represent standard error of the mean.

4.5d Discussion

Nitrogen application in the vineyard after bloom led to increased YAN (Table 4.5-7), without stimulating excessive growth (Table 4.5-4). Nitrogen has been found to stimulate growth of vine canopies (Table 4.2-5, Table 4.2-17, Table 4.2-23, Table 4.2-24, Table 4.2-25)(Bell and Robson, 1999, Candolfi-Vasconcelos et al., 1997, Keller et al., 1999, Kliewer et al., 1991), however the canopy growth response of the vine to nitrogen will vary with timing (Table 4.2-23) (Conradie, 2004, Peacock et al., 1991). Grapes grow in two stages, with a lag phase in between that coincides with veraison (Coombe and McCarthy, 2000). Applying nitrogen after the first growth stage had commenced appears to have little impact on canopy growth.

Leaf health was significantly enhanced by the addition of nitrogen (Figure 4.5-1), however this appeared to have little impact on fruit ripening (Table 4.5-6). Shading will increase with more leaf layers (Table 4.5-3), and increased leaf chlorophyll concentration will further reduce transmission of photosynthetically active radiation (Figure 4.2-11). Skin phenolic compounds will increase with increased exposure, particularly in the first phase of berry growth, and anthocyanin levels are influenced by berry temperature changes due to exposure (Spayd et al., 2002). Research indicates that most anthocyanin production occurs in the early part of post-veraison berry expansion (Fournand et al., 2006). Changes in leaf layer number due to nitrogen addition were not apparent until the 25th of March in this trial (Table 4.5-3). Fruit from this block was harvested on the 24th of March, so the fruit would have been in a similar microclimate for most of the ripening period, and the differences in leaf health would have had minimal impact on phenolic compounds. This was observed with there being no difference in wine tannin content (Table 4.5-9) or phenolic content (Table 4.5-8) between any treatments.

Fermentation rates were strongly linked to the YAN level, regardless of the source and the timing of availability. Timing of DAP addition made no impact on the time taken to finish fermentation in this study. Nitrogen levels during the exponential phase of yeast multiplication have a major impact on the yeast biomass (Beltran et al., 2005). Varela et al. (2004) reported that yeast biomass was the major limitation on fermentation rate in nitrogen deficient ferments, and suggest that early additions will have more impact than later additions. The results of this trial are not consistent with the studies of these authors. Addition of DAP after approximately one third of the sugar had been consumed resulted in a rapid increase in fermentation rate, and after 12 hours the sugar level in ferments receiving the later DAP addition was not different to that of ferments that received DAP prior to inoculation. The YAN of the low nitrogen must in this trial was around 200 mg/l, while Varela et al (2004) and Beltran et al (2005) used deficiency levels of 65 mg N/L and 60 mg N/L respectively. The degree of deficiency may explain some of the differences when compared to the current study. It has been concluded by several studies (various in Bell and Henschke (2005)) that DAP additions around one third of the way through fermentation are preferable to adding all the DAP during the exponential phase of growth since there is a possibility of spikes in ferment temperature, which can lead to stressed yeast. The small scale of the ferments in this trial did not allow significant heat build up, therefore we did not observe any difference in temperature between fermenters. It is possible that DAP adjustments in commercial wineries to correct for relatively minor nitrogen deficiencies may be added at any early stage of ferment with no negative impact on the process, particularly where there is an ability to cool the ferment.

DAP additions pre inoculation were able to increase the wine colour density in this trial. The full rate of DAP resulted in the highest increase in colour, with the only treatment

that was not significantly different being the split DAP addition (Table 4.5-8). DAP additions have been linked to higher colour density in one other trial (Ugliano et al., 2008), where a pre-inoculation DAP addition resulted in an increase in the anthocyanin concentration in the wines. (Bell et al., 2009) reported that increasing nitrogen in the vineyard led to an increase in anthocyanin concentration of wines, with no change in the anthocyanin concentration of the fruit. Both studies indicate that the impact of nitrogen on yeast metabolism or kinetics may be linked to the changes seen, however the mechanisms by which yeast activity may increase anthocyanin concentration and colour density are not clear (Ugliano et al., 2008). Although pre-inoculation DAP additions did have the highest average anthocyanin concentration in this trial, this was not significantly different to other treatments. However, this trend and the results seen by Ugliano et al. (2008) and Bell et al. (2009) indicate that the impact of DAP timing on anthocyanin concentration is worth more investigation. The industry currently recommends additions later in the fermentation process, however if there were more anthocyanin extraction with early additions this may be useful to winemakers.

A significant increase in anthocyanin ionization was observed as a result of pre-inoculation DAP addition and mid-ferment DAP addition. Anthocyanin ionization can be affected by free SO_2 levels, wine pH (Somers and Evans, 1977) or the degree of polymerisation of the anthocyanin. DAP addition can decrease wine pH (Ugliano et al., 2007), which will increase anthocyanin ionization. Increased yeast access to nitrogen can increase the yeast SO_2 production (Osborne and Edwards, 2006), up to at least 44 mg/L total SO_2 concentration. This would be expected to decrease anthocyanin ionization if there remained any significant differences in free SO_2 concentration (Somers and Evans, 1977), however sulphur dioxide may increase anthocyanin extraction (Bakker et al., 1998) if it is present during fermentation. An increase in polymerisation will raise the anthocyanin ionization level (Jackson, 1994). This can be influenced by the phenolic concentration of the must. There was no significant increase in phenolics in the wines from must that received all the nitrogen early in the season, however there was a trend towards increasing phenols and tannins.

Phenolic concentration changes have not been noted by other researchers in response to DAP addition (Ugliano et al., 2008). Bell and Henschke (2005) suggest that DAP additions may lead to changes in extraction through either reduced fermentation times, leading to early pressing and therefore a shortened skin and seed contact period. Tannin extraction is greater under higher ethanol concentrations (Ribéreau-Gayon et al., 2000), therefore if skin contact remains consistent, a wine that finishes ferment sooner will have skins soaking in higher alcohol concentrations for a longer period, which may also increase extraction.

Control wines exhibited a greater hue value than other treatments, indicating a greater degree of oxidation. The reasons for this are unclear. Yeast are active consumers of oxygen, therefore wines with higher biomass may lead to improved oxygen protection. Alternatively, higher DAP fermentations can lead to increased sulphur dioxide production by yeast (Ugliano et al., 2008). SO_2 provides protection from oxidation, however the levels that are required for this are generally significantly greater than the levels produced by yeast.

The results of the MS e_nose analysis and the tasting indicate that there were significant volatile compound differences between the wines tested. The PCA plot of the MS e_nose data indicated that there were clear differences in many of the treatments, although this method does not provide descriptive analysis of the differences involved. The sensory analysis indicated that, while there were differences between the wines, these differences were not great, since there were only two out of all the attributes that showed

any significant differences. The wines from fertilised vines was notably separate from wines from all other treatments, including the control. DAP addition did not appear to alter the MS e_nose results as significantly.

Conclusion

Adding nitrogen in the vineyard between bloom and veraison was able to increase YAN without stimulating further vine growth. Other data from the larger nitrogen trial that this trial was associated with indicates that there would probably be an increase in growth in the following season, however for research purposes this method may be useful to further understand the differences between field applied and winery applied nitrogen.

Nitrogen addition to vines and DAP addition to must both increased fermentation rates. The results of the MS e_nose indicate that there were other impacts on wine volatile properties that resulted from the field nitrogen, that were not achieved by DAP addition. Therefore, it cannot be concluded that the wine quality from YAN increased by fertilisation of vines will be equivalent to wines made with DAP additions to musts.

DAP addition prior to inoculation may have implications for wine colour density. The reasons for this are currently unclear, and more research is required to better understand this phenomenon.

5 General Conclusions and Synthesis

5.1a Research conclusions

Leaf Health and Vine Canopy links

Increasing leaf health, assessed as chlorophyll concentration estimations and retention dates, was linked to greater overall canopy vigour in Pinot Noir and Sauvignon Blanc. In the trials investigating natural changes in vigour and leaf health, growth differences were a result of soil variation (Table 6.2-1, Table 6.2-2, Table 6.2-3, Table 6.2-4, Table 6.2-5, Table 6.2-6), and it could be concluded that the underlying causes of shoot vigour were also influencing leaf health, such that high vigour vines had increased leaf health (Figure 3.2-3). Adjusting the timing of nitrogen application demonstrated that leaf health (Figure 4.2-3) could respond independently of shoot growth (Table 4.2-25). Canopy growth was primarily influenced by conditions up until fruit set, and pre-bloom applications, or later season applications of nitrogen in the preceding season, promoted increased shoot growth. However, leaf health was more reactive to conditions in its growing environment, such as changes in nitrogen status, at any stage of the season. There was little improvement in late season leaf health on vines that received nitrogen prior to bloom and although chlorophyll concentration did increase in the weeks following addition it decreased again to levels of the control vines by the end of the season. In the second season of application, when application timing had no impact on vine growth, there remained a leaf health response with pre-bloom additions continuing to have poor leaf health late in the season. Conversely, leaf retention and chlorophyll concentration could be increased by additions as late as the post veraison timing.

Plant reaction to nitrogen availability can be very rapid, with increased uptake commencing within hours of an increase in soil nitrogen being detected (Britto and Kronzucker, 2005, Forde, 2002). This can stimulate production of cytokinins and increases in chlorophyll concentration, and slow (Gan and Amasino, 1997), or potentially even reverse (Zavaleta-Mancera et al., 1999a, Zavaleta-Mancera et al., 1999b), senescence processes. A nitrogen response in chlorophyll content was observed two weeks after addition of post bloom nitrogen treatments in 2007-08, reflecting this rapid reaction. Therefore, while it may be that leaf health can be used as an indicator of vine vigour in situations where the stresses that limit growth and leaf health are present throughout the growing season, such as when they are linked to the soil environment, this link may not be valid in situations where the factors that can affect leaf health vary within a timeframe that will not affect shoot growth, such as the addition of fertiliser. The implications of this are that it may be possible to maintain good leaf health without producing excessive canopy growth, if timing of stresses can be controlled. While there are a number of studies examining uptake and timing of nitrogen application (Bettiga and West, 1991, Christensen et al., 1994, Conradie, 2001, Holzapfel and Treeby, 2007, Peacock et al., 1991), and also the nitrogen application effects of increasing leaf chlorophyll (Candolfi-Vasconcelos et al., 1997, Keller, 2004, Spring, 2002, Spring and Jelmini, 2002) and photosynthesis (Vasconcelos et al., 2005), there are few reports linking timing of application with different leaf responses.

While leaf health was a useful indicator of vine canopy growth in the vigour gradient trials, other options exist for determining vigour, such as pruning weight measurement (Smart and Robinson, 1991) or infrared imaging (Bramley and Lamb, 2003, Hall et al., 2002). The validity of aerial infrared imaging using the plant cell density index was

confirmed in this trial, on both VSP and Scott Henry trellises. Strong correlations were obtained between PCD and other vine measurements including pruning weight, confirming the research of Dobrowski et al. (2003) and Johnson et al. (2003) on VSP trellises was applicable in cool climate viticulture on a Scott Henry trellis. Vigour level was also well correlated with wine phenolic content and with YAN. This provides useful information towards issues raised by Hall et al. (2002), where the idea of producing maps of quality directly from aerial images. The relationships seen in this study were sufficiently robust to indicate that this is indeed a potential use for the aerial images, although it does require ground data collection also to calibrate the image. The studies here will be of great use in identifying which physical measurements might be valuable to create this map. In particular, the use of leaf health as an indicator of within-season stress, and the use of overall shoot growth as a measure of long-term growing conditions.

Nitrogen additions in warm climates are routinely made after fruit has been harvested, and studies on Pinot Noir in California have indicated that post harvest applications were more effective at increasing vine nitrogen status than applications in spring or summer (Bettiga and West, 1991). However, nitrogen uptake may be reduced when soil temperature is low and leaf health is declining (Keller, 2004), which is often the state in cool climate vineyards after harvest. This trial demonstrated that nitrogen additions after veraison were successful at increasing vine nitrogen status without stimulating new growth. Therefore, nitrogen additions late in the season to improve leaf health without stimulating shoot growth may lead to increased shoot growth in the following season.

Leaf chlorophyll concentration estimates were well correlated to vine nitrogen concentration, and may have a role in diagnosing nitrogen status, as has been suggested by other researchers (Spring and Jelmini, 2002). The responsiveness of leaves to environmental impacts was also clear in this correlation, with increased proximity of leaf nitrogen sampling to chlorophyll estimation date leading to improved correlations.

Leaf Health, Fruit Attributes and Wine Characteristics

In some seasons yield was increased by moderate rates of nitrogen application (20 g N/vine), due to increases in a number of separate components of yield. Bud burst increases at this rage led to more shoots per vine, leading to more bunches per vine. An increase in fruit set in response to nitrogen was implied by results in 2006-07, as has been found by other researchers (Conradie, 2004, Keller et al., 1998), although when this was measured specifically in 2007-08 there was no apparent impact, impact of nitrogen appeared to be strongly influenced by seasonal variation, since there was no change in fruit set in 2007-08.

High rates of nitrogen (50 g N/vine) did not increase yields any further – in fact, they were no different to the control vines after losses due to botrytis fruit rejection were taken in to account. Although it was present, botrytis did not affect the vines treated with 20 g N/vine to the same extent that they did the 50 g N/vine. This may have been a result of increased canopy density reducing air flow and spray penetration (English et al., 1989), or alternatively due to decreased phenolic compounds in the fruit, some of which have antifungal properties (Adrian et al., 2000, Steel and Keller, 2000). Interestingly, botrytis was not well correlated to vigour in the vine vigour trials, even though these vines had increased canopy density and decreased berry phenolic content.

One important outcome of the vigour gradient trials was the observation that correlations between yield, leaf health and vine vigour were not consistent. Yield increases with vigour have been noted in other research (Clingeleffer and Sommer,

1995), and were observed in three of the four trials in this study, however there was no significant correlation between vigour and yield in the fourth trial over three years, reflecting the research of Cortell et al. (2007) on Pinot Noir in Oregon. Yield is often stated as having major links to wine quality. In these blocks, a strong correlation was found between wine tannin concentration and plant cell density (Figure 3.4-5) in block A Pinot Noir, where vigour increased with yield, and also block B Pinot Noir, where it did not. The difference in wine tannin quantity was substantial, with wine from high vigour zones having half or less of the tannin from low vigour zones in both block A Pinot Noir and block B Pinot Noir. It is possible that associations made between yield and quality are examples of increasing yield with increasing vigour, and that it is the vigour that causes the differences in wine quality, not yield *per se*.

Nitrogen addition pre-bloom in 2006-7 was associated with accelerated ripening, although this result was not observed in other seasons. The reasons for this are unclear. It was also associated with an increased colour density as a result of greater anthocyanin ionization. There were indications from fruit chemical analysis that post bloom nitrogen additions were leading to delays in veraison. Until further research is conducted on addition of nitrogen in this period, it is recommended that post bloom applications be used sparingly. There were few benefits from post bloom addition that were not also realised by pre-veraison addition one month later. The same pre-bloom nitrogen treatments that increased juice soluble solids at harvest in 2006-07 were also associated with an increased number of berries that dislodged from the bunch. The analysis of bunch rachises indicated that there might have been an increase in fruit set on these bunches, potentially increasing berry counts per vine and creating crowding between the berries. Fruit set increases with increased nitrogen have been observed by other authors in Pinot Noir (Keller et al., 2001a).

Vines with low leaf health tended to produce fruit with low YAN concentration but increased phenolic concentration, whether the leaf health differences resulted from nitrogen deficiency or soil variability. The correlations of leaf nitrogen concentration and leaf chlorophyll to YAN demonstrated in the nitrogen timing by rate trial indicate that YAN is sensitive to vine nitrogen conditions. Other research has also noted the strong correlation between YAN and overall vine nitrogen status (Bath et al., 1991, Kliewer and Cook, 1974). This relationship holds true in reverse also – vines that display nitrogen deficiency symptoms may well have reduced YAN, and in extreme cases may not have sufficient YAN for fermentation purposes. However, it was clear from the YAN concentration of the Sauvignon Blanc vines in block C that a vigorous canopy was not a guarantee of high YAN concentration, with the YAN levels in the juice from high vigour vines still being below the threshold of 140 mg N/L that is suggested to ensure a successful ferment. There are varietal differences in nitrogen uptake (Treeby et al., 1998), however whether this is a result of varietal differences or a feature of that site is unclear, although soil nitrate levels were very low in the soil tests from block C (Table 6.2-1).

Moderate nitrogen addition rates (17 g N/vine) at veraison in 2005-06 were associated with a decrease in YAN. This apparently contradictory response was not observed in other trials, and may be a phenomenon that occurs only when enough nitrogen is made available to slow leaf senescence processes, however not enough to serve the needs of both the leaves and the fruit. In this situation fruit may be worse off, since they miss out both on the fertiliser-sourced nitrogen, and also from nitrogen that would have otherwise been remobilised from the senescing leaves. After veraison fruit receive little from the xylem, with phloem being the main channel connecting the fruit to the rest of the vine (Keller, 2010). Nitrogen will travel as nitrate and amino acids from the roots via the xylem to the leaves, where nitrate reductase activity will be strongest. Therefore, if the

leaves utilise all the fertiliser nitrogen, there will be none for the fruit. It may be that the leaves of vines receiving 17 g N/vine locked up all nitrogen in leaves, preventing export, while 51 g N/vine was enough to provide nitrogen for phloem flow.

While there was obvious seasonal variability and also local differences in YAN levels, it was clear from block A Pinot Noir that the patterns of difference were constant across seasons (Figure 3.3-3). This reflects other studies where the boundaries between vigour zones within vineyards will often remain stable from year to year (Bramley and Lamb, 2003, Hall et al., 2002). This suggests that management of YAN levels may benefit from historical YAN data linked to blocks or zones of vigour with a vineyard. Vines that produce high YAN grapes are likely to consistently produce high YAN grapes, and vice versa. This may then direct management activities, such as nitrogen fertilisation.

YAN adjustment in the vineyard produced different wines to those produced when adding diammonium phosphate (DAP) to fermenters in the winery, although DAP was also successfully able to increase fermentation rates (Figure 4.5-2). DAP added prior to inoculation also increased wine colour density through an increase in anthocyanin ionization. The reasons for this are unclear, however other recent studies have indicated that yeast activity may impact anthocyanin concentration when altered by DAP addition (Ugliano et al., 2008). In that study, it was suggested that temperature increases associated with more rapid yeast metabolism may also influence the release of anthocyanins into solution, however there was no evidence of increasing temperatures in these trials.

Leaf health was also linked to wine phenolic concentration, which has a major impact on wine quality. This was apparent in the sensory analysis from block A Pinot Noir, where tasters could clearly identify the lower tannin in the high vigour wine. However, Pinot Noir quality is also strongly influenced by wine aroma, and the high vigour wine did not score lower than the low vigour wine. It may be that tannin concentration is not as relevant a measure of quality in Pinot Noir as it is in other varieties, although colour and anthocyanin concentration have been correlated with quality in Beaujolais wines, a closely related vine to Pinot Noir (Jackson et al., 1978). There are currently no well developed analyses techniques for aroma in Pinot Noir, although some are being developed (Fang and Qian, 2005, Fang and Qian, 2006). Research into Sauvignon Blanc wines has demonstrated increasing concentration of important volatile compounds in response to nitrogen application (Choné et al., 2006, Peyrot des Gachons et al., 2005), and while there is no current information on Pinot Noir volatile compounds, such data would greatly increase the understanding of wine quality and leaf health links.

5.1b Conclusions

These trials showed clearly that leaf health can influence wine quality directly, through changes in exposure of fruit, which in particular affect phenolic compounds and tannins, and also with potential increases in ripening with increased leaf health in some seasons. There were many indirect links also, such as through a general link to vigour, and then to YAN concentration.

Outcomes of the trials indicate that moderate vigour and leaf health are likely to meet the best balance of yield against quality. Lower vigour vines may have decreased yields, and also wines with higher tannins and colour density, however addition of moderate rates of nitrogen was able to increase yield with minimal change in wine spectral measurements or wine tannins. Even so, wine quality in Pinot Noir was hard to definitively ascribe to one vigour level, as demonstrated by sensory analysis.

The trials pave the way towards increasing the use of leaf health as a monitoring tool to

help judge wine potential. Such systems have been suggested in the past (Smart and Robinson, 1991), but stand to be significantly updated by using remote sensing and an increased understanding of the links between canopies and wines.

5.1c Implications for management

Increasing vigour led to consistent reductions in wine tannin and phenolic concentration. This may have a major impact on wine quality, but the consistency of the relationship makes management of different zones of vigour more practical, with options such as segregated harvests or different canopy management zones being possibilities. These trials demonstrated that aerial imaging using near infrared wavelengths to generate a vigour index was able to discriminate between different vigour zones. Further, vines from these zones were shown to produce wines that were significantly different in a number of spectral and sensory aspects. The project also demonstrated that leaf health may also be used as an indicator of vine vigour differences, which can allow rapid conclusions to be drawn in the field, when aerial images are not available.

This makes it possible to construct management zones based on the vine vigour. Grapes may then be harvested separately, or management in each zone may be made more appropriate for the level of vine vigour of that zone. There is a wide range of options for managers to implement, and the most appropriate actions will be determined by the site, wine style and grape variety. Describing all the options is outside of the scope of this thesis.

Vineyards with poor late season leaf health may be displaying symptoms of nitrogen stress. This can be determined using both tissue tests as well as physical observation of the vines. Signs of low nitrogen include early leaf senescence, low leaf chlorophyll levels, reduced bud burst and reduced shoot vigour. Nitrogen concentration of the fruit may also be reduced, and this can be associated with sluggish or stuck fermentations. Addition of nitrogen can also increase bud burst when it is limiting. Areas in a vineyard that have a nitrogen stress in one season may expect to be affected by nitrogen stress in most seasons unless action is taken to increase nitrogen availability. Soils may not deliver enough nitrogen to vines with either soil nitrogen or organic carbon contents are low. Nitrogen is removed every season from the vineyard in the fruit, and this must be replaced by soil reserves or new inputs. Conradie (1991) estimated that harvest led to a nitrogen removal of around 1.6 kg nitrogen per ton of fruit harvested, while Löhnertz (1991) estimated loss to be from 1.4 to 2.2 kg nitrogen per ton. Note that Löhnertz also states that an organic carbon content of over 2.5% is linked to a soil that can provide most vine nitrogen requirements, while other authors suggest that legumes in the inter-row may supply sufficient nitrogen for the vine's requirements. Competition from plants on the floor of the vineyard may also reduce nitrogen availability. Several trials have demonstrated that cultivation or herbicide removal of inter-row vegetation can increase vine vigour (Morlat and Jacquet, 2003, Wheeler et al., 2005).

Published nitrogen recommendations suggest that rates around 20-50 kg N/ha are suitable for viticulture (Robinson, 1992, Smart et al., 1986, Weaver, 1976). Bell and Robson (1999) observed increases in canopy growth with additions up to 100 g N/vine, but no further increase up to 400 g N/vine. Application of 20 g N/vine is equivalent to a rate of 59 kg N/ha, while 50 g N/vine is equivalent to 148 kg N/ha, at the vineyard spacing in the trial block (2.25 m row spacing x 1.5 m vine spacing; 2963 vines per hectare). The yields in these trials ranged from 3.2 to 4.5 kg/vine, which would translate to a replacement requirement of 5-7 g N/vine based on the estimates presented above. The actual amount will vary however, as was evident in the variation in YAN content.

The results in these trials support the published recommendations. Applications of nitrogen at 20 g N/vine to a site that was displaying nitrogen deficiency symptoms improved leaf health and increased yields, while having little impact on wine spectral

assessments. YAN levels were also improved by this rate of addition, from around 150-160 mg N/L, close to the minimum recommended YAN of 140 mg N/L to 220-240 mg N/vine (Figure 4.3-6, Figure 4.3-7). Additions of 50 g N/vine increased disease risks (Table 4.3-25) and produced wines with less tannin than the lower application rates. Yields were no higher than on the control vines, suggesting that high rates of nitrogen should be avoided. Other potential changes from adding nitrogen include increased fruit set in some seasons, more rapid ripening in some seasons.

An alternative method of increasing nitrogen availability is to increase the soil carbon content, which may allow a greater degree of mineralisation of soil nitrogen, leading in turn to more nitrogen available to plants.

Nitrogen application timing can be used to influence vine leaf health, although it only influenced vine shoot vigour in the first season of application, when pre-bloom nitrogen additions stimulated shoot growth. Nitrogen availability in the early part of the season can determine the shoot vigour, with much less impact on shoot growth from increased nitrogen within the vine after fruit set. Nitrogen will be carried over from one season to the next, and is actively cycled within the vine from storage organs at the start of the growing season, to the leaves, fruit and shoots as required by their growth demands, and back to storage in woody tissues from leaves at the end of the season. Therefore, applications late in the season will be stored for remobilisation in the following season, as was observed in bloom leaf tests in this study. Applications of nitrogen post veraison were observed to increase late season leaf health and also increase the following season shoot growth. No increases in YAN were observed post veraison, indicating that all nitrogen that was taken up by the vine was used to maintain leaves and for storage. These results suggest that post veraison applications in cool climates may offer similar benefits to vines provided by post harvest applications in warmer climates.

A proposed set of actions to help determine optimum nitrogen management, based on this study and other research, would be:

- Diagnose nitrogen status by combining observations of leaf health as chlorophyll content and timing of senescence, vine vigour, and leaf analysis.
- If a deficiency is indicated, add moderate rates of nitrogen to vines, being aware that there will be implications for the following season.
- Add nitrogen prior to bloom to increase fruit set.
- Add nitrogen after veraison to avoid delays in veraison development, and to maintain improved late season leaf health.
- Monitor results through changes in leaf colour and must YAN.
- Reassess the following season.
- Be aware that nitrogen deficient areas in a vineyard may consistently have issues. However, seasonal nitrogen availability will vary, which can impact all areas of a vineyard.
- Consider improving soil health in areas that show consistent nitrogen deficits, by increasing soil carbon. Be aware that soil carbon levels in the inter-row may not represent the soil health as it relates to the vine, due to competition with other plants.

6 Appendix

6.1 Fermentation management: an investigation of small research scale punch down and submerged cap systems

6.1a Introduction

Fermentation of dark skinned grapes to produce red wines involves extraction of compounds from the skins and seeds of grapes, with phenolic compounds such as tannins, flavan-3-ols and anthocyanins being of major importance to wine quality and colour. A number of methods have been utilised to approximate the winemaking process in research, so that conclusions may be drawn on how changes in grape attributes impact the wines. These often utilise extraction in ethanol, and may involve separation of skins from the seeds, or homogenised samples.

Small-scale winemaking has the potential to provide an extraction that is more comparable to the extraction that occurs in commercial wineries. However, there are multiple options for management of red wine fermentations, including punch-down ferments, or submerged cap ferments. Floating grapes will form a "cap" in a ferment, and in a conventional ferment this cap will be regularly immersed in fermenting wine to increase extraction and maintain even temperatures in a ferment. The cap management system can impact extraction of phenolic compounds,

Small-scale fermentation management options must deal with a number of specific issues. Oxidation is a risk in smaller ferments, since the surface area to volume ratio may easily be increased by disturbances of the ferment above what may occur in a large volume of wine. Oxidation can change wine colour parameters (Somers and Evans, 1977) and organoleptic qualities (Jackson, 1994). Conversely, ferments that are carried out with too little oxygen availability may have an increase in "reductive" odours, such as hydrogen sulphide (Jackson, 1994). Consistency between ferments is also important in research, to allow statistical analysis of the wine attributes by reducing variability between treatment replicates. Large trials may involve many individual ferments. A system that minimises risk of spoilage or variability, while needing minimal inputs per ferment is required.

Punch-down systems are commonly used in commercial wineries. This system involves pushing the floating cap into the juice below using a plunger. Punch-down ferment have the advantage of regular exposure of the ferment to oxygen, which can assist in ferment health and remove unwanted volatile odours such as hydrogen sulphide.

Submerged cap systems are not often used commercially, however they have the advantage of lower labour inputs compared to punch-down management. They involve using a heading down board to hold the cap under the surface of the liquid for the duration of the ferment. However, these systems have potential problems, such as insufficient oxygen availability, and stratification of juice leading to difficulties in monitoring ferments.

This trial was established to investigate differences between submerged cap and punch down ferments, and compare both to commercial practice. Further, the use of aeration in submerged cap ferments was assessed. This knowledge will allow more information on the relative suitability of each or the research-scale processes for investigating wine quality. Further investigation was made to determine the degree of within-ferment

variability in the submerged cap system.

6.1b Materials and Methods

Experiment 1 - Punch down compared to Submerged cap ferment management systems

Pinot Noir grapes from a vineyard in northern Tasmania were used for the trial. Grapes were obtained from commercially hand-harvested fruit destined to go into a single-lot ferment. Fruit was selected from a range of fruit bins, to make it as representative as possible.

The fruit was chilled overnight and then crushed into 20L plastic containers for fermentation using a Baesso 80kg crusher/destemmer unit (Australian Winemakers). The must was divided among nine fermenters, ensuring each vessel contained a homogenous sample, with 12 kg of must per fermenter. Potassium metabisulphite was added at a rate that provided 50 ppm of free SO_2 to the must, and mixed through. Lafase HE enzymes (Laffort, Bordeaux) were added at a rate of 30 ppm and the must then transferred back to the cool room overnight.

The next day, the must was warmed to 20 °C and inoculated with 250ppm of Lalvin Assmanshausen yeast (Lallemand, Canada), rehydrated according to the manufacturers recommendations. Yeast was mixed through the must, and from then all treatments were kept at 25ºC.

The day following inoculation, fermenters were randomly allocated to each treatment, each with three replicates (Table 6.1-1).

Table 6.1-1 Description of the treatments used to compare submerged cap ferments and punch down ferments

Treatment	*Description*
Punch Down	Cap punched down twice a day, with 20 plunges per bucket
Submerged cap + aeration	Cap submerged until the end of the ferment, with an aerative transfer and return on day 2
Submerged cap, no aeration	Cap submerged until the end of ferment

Heading down boards were constructed from plastic discs, drilled with a series of 8mm holes, and these were used to hold down the fermenting cap of skins. They were held in place using stainless steel rods.

Aeration of the submerged cap + aeration treatments was carried out by pouring the fermenting juice from the fermenter, into a separate 20L container, and then returning it to the fermenter. This was done once at 48 hours post inoculation.

Must density measured daily as Baumé was used to follow the progress of the fermentation, using an Anton Paar 35m density meter. Ferments were pressed off when Baumé's were below 0.5, indicating that most of the sugar had been consumed. It was carried out after punch downs on the punch down treatments, and sampled directly from the juice above the submerged cap in the remaining ferments. Pressing was carried out in a water bag basket press, with a maximum pressure of 200kpa. Wines were protected from air using dry ice, and were pressed into 3 L PET containers (Caled Containers, Tasmania, Australia). Excess air was removed from the bottles to minimize oxidation.

At 96 hours post inoculation, samples were taken from above and below the heading down board, without disturbing the cap. The ferment rate measurements taken at this time were converted to proportion of sugar fermented by dividing the Baumé at that

time with the total range of Baumé measurements across the trial for each fermenter. The coefficient of variation for each treament was then calculated, to assess the variability in ferment rate between treatments.

Wines remained in a 25ºC room until all were finished fermenting (assessed using Clinitest tablets (Ames Division, Miles Laboratories, Illinois)). At this point they were transferred to a 2ºC room. Following settling of solids, the clear wine was racked by siphon into fresh 3L PET containers, and potassium metabisulphite added to provide 50ppm of free SO2. They remained at 2ºC until sampled for analysis.

The wine produced in the commercial winery (Tamar Ridge Estates, Tasmania) was made using standard winery practice, combining pumping juice over the cap and punch-down cap management. A sample was sourced at the time of racking into barrel. 3 L was taken and transferred to the 2ºC room. Its treatment from that time was identical to the other wine treatments.

Wine analysis included a modification of Somers analyses developed by the AWRI (Mercurio et al., 2007, Somers and Evans, 1977), and also the Dambergs wine tannin analysis method developed by the AWRI (pers. comm.., Dr R. Dambergs, AWRI). Statistics were analysed using GenStat software.

Experiment 2 – Fermentation dynamics in a Submerged Cap fermenter

Two batches of Pinot Noir must approximately 12 kg each were prepared for fermentation as in experiment 1. The ferments were treated identically, and were called fermenter 1 and fermenter 2.

The day following inoculation, ferments were mixed to ensure even distribution of the yeast, and then submerged caps fitted to the ferments. Modifications had been made to the submerged cap heading down boards to allow wine samples to be taken from both within the cap, and in the free juice below the cap. These used flexible plastic piping, connected to the intake end of a plastic 3mL Pasteur pipette. The bulb section of the pipette had a large number of holes, designed to prevent the intake of berries and seeds. A 60mL syringe was then connected to the other end of the pipe, allowing fermenting juice to be drawn up. This was deposited in a beaker until enough sample had been taken to allow measurement of Baumé and temperature, using the Anton Paar 35m density meter.

Ferments were tracked until pressing, recording Baumé above the cap, within the skins and in the free juice below the cap.

A single Baumé reading of the submerged cap ferments in experiment one was taken from above and below the cap 96 hours after inoculation, to compare the differences with a larger sample set. Juice was taken from below the cap using a syringe with a 5 mm plastic hose.

6.1c Results

Experiment 1 – Punch down compared to Submerged Cap

There were no observed differences in the fermentation rates from either cap management system, or from aeration of submerged cap ferments Figure 6.1-1). When the wines were pressed off the skins after fermentation was nearly complete, the submerged cap ferments had a higher Baumé than the wines from the punch down

ferments (Table 6.1-2).

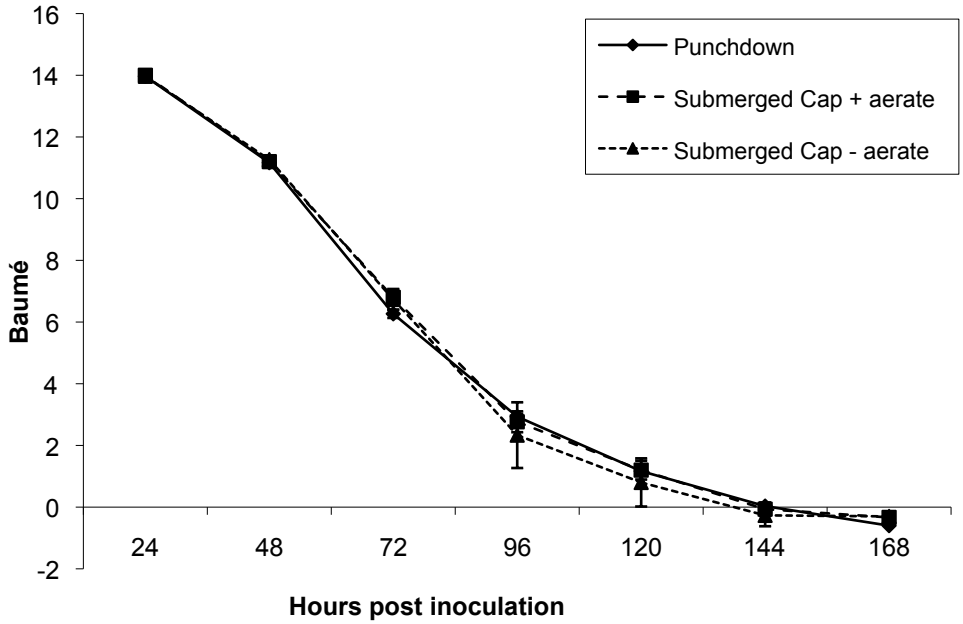

Figure 6.1-1 Must density drop following inoculation under three different cap management systems. Error bars are +/- SEM.

*Table 6.1-2 Baumé levels after pressing ferments off skins, from three different cap management systems. (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Treatment	*Post Pressing Baumé*
Punch-down	-0.6a
Submerged Cap + aeration	-0.3b
Submerged Cap – aeration	-0.3b
Significance	*

Baumé levels above and below the cap 96 hours post inoculation showed substantial variation in the submerged cap ferments in experiment 1. The differences in Baumé above and below the heading down board were up to 2.9 (Table 6.1-3), however the Baumé from below the cap was less than above the cap in three cases, and more than the juice above the cap in three other cases.

Table 6.1-3 Must density level at 96 hours post inoculation above and below the heading-down board of ferments used in Experiment 1.

Treatment	*Rep*	*Baumé above board*	*Baumé below board*	*Difference (Above – Below)*
SC1	1	3.0	1.8	1.2
SC1	2	3.2	0.4	2.8
SC1	3	2.1	2.5	-0.4
SC2	1	2.8	2.9	-0.1
SC2	2	0.3	3.2	-2.9
SC2	3	3.9	2.7	1.2

Sugar levels 96 hours post inoculation did not vary in their mean (Figure 6.1-1), however the coefficients of variation for submerged cap ferments was greater than in the punch-down ferments (Table 6.1-4). Samples from on top of the heading down board and from

underneath also showed increases in variability.

Table 6.1-4 Coefficients of variation of the proportional drop in soluble solids at 96 hours post inoculation within different cap management systems, and different positions within the ferment.

Treatment	*Coefficient of Variation*	*C.V. of under-cap results*
Punch-down	0.04	n.a.
Submerged Cap + aeration	0.17	0.62
Submerged Cap, nil aeration	0.73	0.07

Wine from punch-down ferment management systems had significantly less anthocyanin, tannin, total phenolics and total pigments than the wines made using the submerged cap method (Table 6.1-5). This was associated with an increase in colour density in the submerged cap ferments wines. There was also a slight increase in hue value in the punch-down ferments.

The aeration of the ferments appeared to have little impact on fermentation rate (Figure 6.1-1), however it did slightly but significantly increase the wine hue value.

*Table 6.1-5 Somers and tannin (Dambergs) analysis results from different methods of fermentation management. PD = Punch Down; SC1 = Submerged cap + aeration; SC2 = Submerged cap – aeration; Com = commercial winery wine from the same batch of grapes (not used for statistical analysis since there was only a single sample). (Significances: * P ≤ 0.05; ** P ≤ 0.01; *** P ≤0.001; n.s. = no significant difference)*

Ferment management	*Chem age 1*	*Chem age 2*	*Total Anthocyanin (mg/L)*	*Anthocyanin ionization %*	*Colour density*	*Colour density SO_2 corrected*	*Hue*	*SO_2-resistant pigments*	*Somers phenolics (AU)*	*Tannin (g/L)*	*Total pigments*
PD	0.21	0.045	227a	12.7	3.38a	4.23a	0.69b	0.56	23.7a	0.45a	12.3a
SC1	0.20	0.043	272b	13.2	4.10b	5.06b	0.68b	0.64	29.2b	0.65b	14.7b
SC2	0.20	0.041	280b	13.5	4.19b	5.06b	0.67a	0.62	28.9b	0.63b	15.0b
Sig	n.s.	n.s.	**	n.s.	**	**	*	n.s.	**	*	**
Com	0.21	0.049	282	16.1	4.98	5.70	0.64	0.76	37.2	1.10	15.4

Wines made with punch-down cap management had greater coefficients of variation than submerged cap ferments for chemical ages 1 and 2, and SO_2-resistant pigments. Total phenolics and tannin levels were less variable than the submerged cap ferments (Table 6.1-6).

Table 6.1-6 Coefficients of variation (relative percentage of the standard deviation) for Somers measures and wine tannins (Dambergs') using different methods of fermentation management. PD = Punch Down; SC1 = Submerged cap + aeration; SC2 = Submerged cap – aeration.

Ferment management	Chem age 1	Chem age 2	Total Anthocyanin (mg/L)	Anthocyanin ionization %	Colour density	Colour density SO_2 corrected	Hue	SO_2-resistant pigments	Somers phenolics (AU)	Tannin (g/L)	Total pigments
PD	11.7	12.9	3.7	2.1	0.7	2.0	0.8	12.0	1.1	4.9	1.2
SC1	8.2	8.4	4.0	2.6	3.1	3.1	1.7	8.7	3.5	8.9	2.5
SC2	4.4	5.3	0.1	6.7	6.4	4.6	0.5	6.1	14.2	6.5	5.8

There was a significant correlation above the cap between Baumé at 96 hours post inoculation and tannins in the submerged cap ferments, however there was no significant correlation with the Baumé measured within the cap.

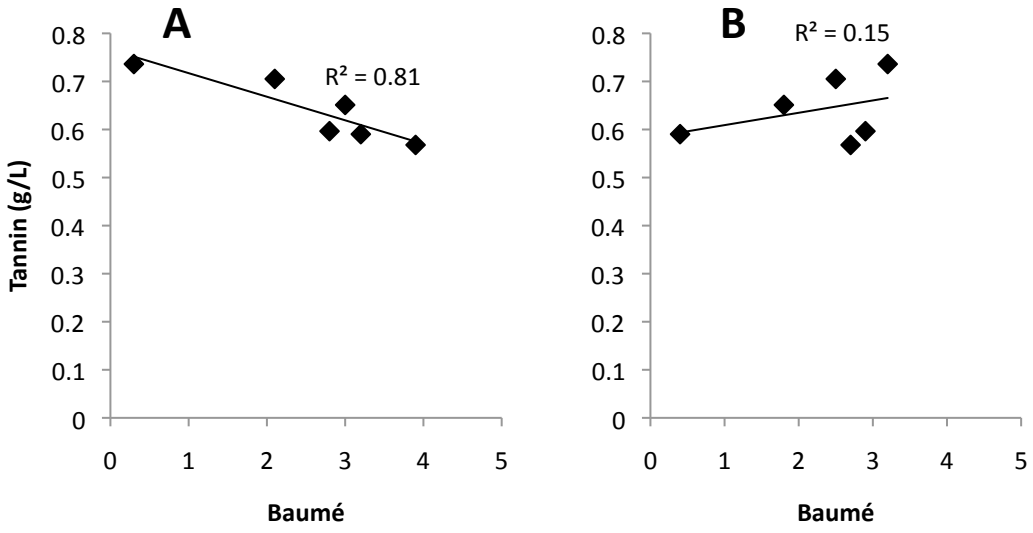

Figure 6.1-2 Wine tannin concentration (g/L, Dambergs' method) in submerged cap ferments against Baumé levels above the heading down board (A) and below the heading down board (B)

Experiment 2 – Fermentation dynamics in a Submerged Cap fermenter

Fermentations were noticeably different at different locations in the fermenter (Figure 6.1-3). In fermenter 1, the juice within the cap fermented faster than the juice above the cap, with the juice below the cap being the slowest of all. In the other fermenter, this was the case up until 72 hours post inoculation. At this stage, the juice within the cap jumped up to be much more similar to the juice below the cap in fermentation rate.

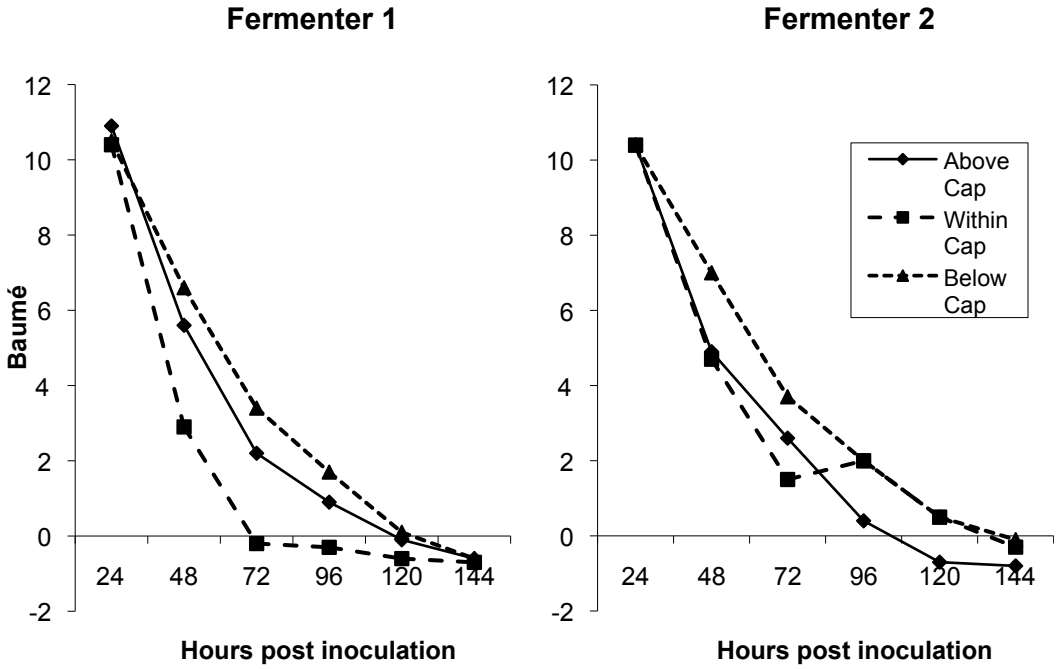

Figure 6.1-3 Fermentation progression above the submerged cap, within the skins (within cap) and in the free juice below the skins (below cap), for two fermenters.

6.1d Discussion

Wines made using a submerged cap method showed significantly more extraction of phenolics and anthocyanins than those made by the punch-down method (Table 6.1-5). Anthocyanin extraction can be increased by longer soaking times prior to inoculation or slow starts to fermentation, and physical breakage of grape skins (Ribéreau-Gayon et al., 2000). Although punch-down ferments provided more physical agitation of the grape skins, the submerged cap ferments were still higher in total phenolics and tannins. Keeping the cap submerged had the effect of maximising skin to juice contact, which may have led to the increased extraction. The greater decrease in Baumé within the cap in submerged cap ferments (Figure 6.1-3) may have been linked to a more rapid increase in alcohol concentration in the ferment medium immediately surrounding the grapes. However, in the submerged cap ferments wine tannin was negatively correlated to the Baumé level at 96 hours post inoculation above the cap, but not below the cap in the zone where skin contact is increased (Figure 6.1-2). Alcohol levels can increase phenolic extraction (Harbertson et al., 2009), although do not increase anthocyanin extraction (Ribéreau-Gayon et al., 2000).

Tannin concentration and total phenolic concentration of wines from both small-scale ferment methods was lower than in the commercially produced wine (Table 6.1-5). Anthocyanin concentration in the wines from punch-down ferments was also lower than the level of anthocyanin in the commercial wine, however anthocyanin concentration did not vary greatly between the commercial wine and the submerged cap ferments (Table 6.1-5). Sampaio et al. (2007) found that small lot winemaking using a submerged cap extracted significantly less seed tannins than a commercial ferment, however skin tannin levels were not different by the end of the ferment. The time grape solids are in contact with the liquid of the ferment can influence extraction of both phenolics (Budić-Leto et al., 2008) and anthocyanins (Bosso et al., 2009, Gordillo et al., 2010, Puertas et al., 2008), and Sampaio et al. (2007) suggest that higher temperatures during fermentation and

longer maceration times may be used to make small-scale wines more similar to commercial wines.

There appeared to be little benefit from an oxidative aeration during the fermentation of submerged cap wines on fermentation rates. The researchers detected no hydrogen sulphide in these trials, however the wines were not chemically analysed for H_2S. The aerative oxidation did lead to an increase in hue values, indicating that there was some oxidation of the wines (Somers and Evans, 1977) as a result of the aeration, however this was minimal. Punch-down ferments also increased wine hue.

The submerged cap wines exhibited a greater degree of within-ferment variability in fermentation rate compared to the punch-down ferments. Fermentation rate was particularly high within the skins (Table 6.1-3, Figure 6.1-3), possibly because of increased availability of nitrogen and other nutrients within the skins and seeds (Stines et al., 2000). There was clear stratification both above and below the heading down board, and also between must within the grape skins and must below the skins (Figure 6.1-3), however this appeared to be less stable, therefore mixing may not require excessive disturbance of the ferment (Figure 6.1-3B). There was also considerable variability in fermentation rate and the within-ferment variability between the submerged cap ferments (Table 6.1-4, Figure 6.1-3). Punch-down ferments appeared to be more consistent in extraction of tannins and total phenolics than submerged cap ferments. The correlation between ferment progress and wine tannin in the submerged cap ferments (Figure 6.1-2) indicates that the variability in ferment rates between submerged cap wines may have led to an increase in variability in wine quality. Maintaining consistency between ferments therefore will be required to ensure low variability between treatments.

Potential actions to increase consistency of submerged cap ferments could be made in both fermenter design, and ferment management. The round holes used in the heading down board in this trial were observed to block easily with grape skins, limiting the movement of juice and gas from one side of the heading down board to the other. Altering the shape and/or arrangement of the holes in the heading down board may increase the movement of gasses and liquids, reducing within-ferment variability. Alternatively a manual mixing of the juice may be used to reduce within-ferment variability within submerged cap ferments.

6.1e Conclusion

This trial indicates that small-scale winemaking can be a useful tool for research, although to replicate the equivalent levels of extraction to commercial wineries may take modifications to the systems used.

Submerged cap ferments appear to be a step towards this aim, since they provide greater extraction than punch-down ferments, and are less labour-intensive. The fermenter design used in this trial may require modification to optimise its use in research to reduce between-ferment variability. Fermentation variation appeared to increase the variation of extraction of phenolics.

Care should also be taken if ferment rate monitoring is to be carried out, in case of within-ferment variability, which may make effective monitoring of juice difficult. Options such as measuring weight loss from evolved CO_2 may be a better method for monitoring submerged cap fermentations.

6.2 Soil analyses

All soil analyses were collected by Sam Rees from the University of Tasmania as part of his PhD project (currently under review). Analysis was carried out by CSBP laboratories (Bibra Lakes, WA).

Table 6.2-1 Block C-07 Sauvignon Blanc vigour trial in 2006-07 soil analysis - Major nutrient concentrations of different horizons from two sites

	Horizon	Depth (cm)	NITRATE N mg/kg	AMMONIUM mg/kg	PHOS mg/kg	POTASSIUM mg/kg	SULPHUR mg/kg	ORGANIC CARBON %
Low vigour	A11p	0-10	6	2	92	181	7.1	1.45
	A12	10-26	1	2	16	86	10.8	1.46
	A2/B1	26-36	1	2	4	47	22.8	0.65
	B21	36-60	1	1	1	70	57.3	0.48
	B22	60-85	1	1	-1	61	67.4	0.27
	B23	85-115	1	2	-1	63	48.8	0.3
	B24	115-140	1	2	1	55	36.7	0.22
High vigour	A11	0-20	11	4	68	625	14.3	4.05
	A12	20-40	16	12	19	136	25.1	3.48
	A2/B1	40-58	6	3	9	55	14.5	2.11
	B21	58-90	2	2	2	128	63.5	0.51
	BC	95-115	1	2	2	86	44.9	0.35
	D	115-135	1	2	2	74	40.7	0.33

Table 6.2-2 Block C-07 Sauvignon blanc vigour trial in 2006-07 soil analysis – conductivity and pH of different horizons from two sites

	Horizon	CONDUCTIVITY dS/m	pH $CaCl_2$	pH H_2O
Low vigour	A11p	0.091	6.9	7.5
	A12	0.059	6.4	7.1
	A2/B1	0.073	4.9	5.7
	B21	0.047	4.8	5.4
	B22	0.058	4.8	5.5
	B23	0.063	4.7	5.4
	B24	0.075	4.4	5
High vigour	A11	0.122	6.3	7
	A12	0.13	4.8	5.5
	A2/B1	0.044	4.7	5.6
	B21	0.068	4.2	5
	BC	0.077	4.2	4.8
	D	0.08	4.1	4.9

Table 6.2-3 Block C-07 Sauvignon Blanc vigour trial in 2006-07 soil analysis - Micronutrient concentrations and exchangeable cations of different horizons from two sites

	Horizon	Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	Exchangeable Ca meq/100g	Exchangeable Mg meq/100g	Exchangeable Na meq/100g	Exchangeable K meq/100g
Low vigour	A11p	4.17	1.37	6.57	32.74	6.88	2.06	0.11	0.48
	A12	0.4	0.23	6.11	50.34	6.19	2.01	0.15	0.22
	A2/B1	0.32	0.16	1.53	120.66	1.41	1.04	0.1	0.11
	B21	0.17	0.18	0.21	16.49	2.33	3.31	0.19	0.19
	B22	0.07	0.11	0.2	8.42	2.26	7.47	0.39	0.17
	B23	0.12	0.11	0.05	7.6	1.63	10.78	0.74	0.18
	B24	0.17	0.11	0.05	3.76	1.11	10.6	0.93	0.13
High vigour	A11	3.24	4.71	21.21	62.15	16.68	7.32	0.21	1.59
	A12	0.93	0.56	65.32	156.9	6.74	2.34	0.22	0.21
	A2/B1	0.69	0.36	11.79	189.79	2.26	1.07	0.14	0.15
	B21	0.23	0.16	1.03	17.36	2.57	6.62	0.31	0.34
	BC	0.07	0.1	0.3	4.22	2.21	7.68	0.46	0.19
	D	0.1	0.13	0.2	3.73	1.76	9.25	0.54	0.17

Table 6.2-4 Block A Pinot Noir soil analyses – major nutrient concentration of different horizons from two sites

	Horizon	Depth (cm)	NITRATE N mg/kg	AMMONIUM mg/kg	PHOS mg/kg	POTASSIUM mg/kg	ORGANIC CARBON %
Low vigour	A11	0-10	23	7	30	471	3.99
	A12	10-22	9	7	13	190	3.88
	B21	22-42	1	4	4	83	1.48
	B22	42-60	1	2	3	28	0.65
	B23	60-90	6	2	2	27	0.45
	B24	100-130	1	1	2	c	0.24
High Vigour	A11	0-7	53	8	59	644	3.78
	A12	7-18	31	9	17	648	3.49
	A3	18-28	5	3	8	389	1.39
	B21	28-55	5	2	4	84	0.5
	B22	60-95	1	1	3	74	0.29
	B23	95-120	1	1	4	50	0.27
	B24	120-135	1	2	3	46	0.19

Table 6.2-5 Block A Pinot Noir soil analyses - pH and conductivity of different horizons from two sites

	Horizon	***CONDUCTIVITY dS/m***	***pH $CaCl_2$***	***pH H_2O***
Low vigour	A11	0.18	6.2	7.1
	A12	0.24	5.8	6.6
	B21	0.11	5.3	6.1
	B22	0.25	4.8	5.3
	B23	0.39	4.9	5.5
	B24	0.45	4.7	5.2
High Vigour	A11	0.17	6.4	7.1
	A12	0.12	6.1	6.8
	A3	0.09	5.6	6.4
	B21	0.16	5.9	6.5
	B22	0.18	6	6.5
	B23	0.02	5.6	6.2
	B24	0.30	5.7	6.1

Table 6.2-6 Block A Pinot Noir soil analyses - micronutrient concentration of different horizons from two sites

	Horizon	***Cu mg/kg***	***Zn mg/kg***	***Mn mg/kg***	***Fe mg/kg***	***Exchangeable Ca meq/100g***	***Exchangeable Mg meq/100g***	***Exchangeable Na meq/100g***	***Exchangeable K meq/100g***
Low vigour	A11	4.04	2.09	21.12	33.53	15.93	3.4	2.08	1.2
	A12	1.84	1.11	27.49	39.86	10.4	3.79	2.12	0.5
	B21	0.94	0.12	11.27	19.33	3.25	3.6	2.15	0.21
	B22	0.35	0.13	4.28	7.16	1.05	3.3	2.17	0.07
	B23	0.4	0.14	0.6	11.58	0.91	8.52	2.94	0.06
	B24	0.24	0.1	0.53	3.75	0.56	8.88	3.45	0.08
High Vigour	A11	8.74	3.97	33.35	27.5	17.3	7.94	0.34	1.64
	A12	3.25	0.94	36.5	32.46	11.95	7.08	0.24	1.64
	A3	1.41	0.24	21.69	17.88	3.77	2.83	0.39	0.96
	B21	0.5	0.5	7.18	6.49	2.08	4.11	1.26	0.18
	B22	0.26	0.62	1.71	3.51	1.68	3.95	1.28	0.17
	B23	0.34	0.51	1.39	0	1.87	5.07	1.8	0.11
	B24	0.22	0.44	0.85	2.32	1.89	5.24	2.11	0.1

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